The Symposium

on

THYRO-GONAD-ADRENAL-PITUITARY RELATIONSHIPS



National Institute of Sciences of India

New Delhi

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on October 2, 3, 4, 1959



National Institute of Sciences of India

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PREFACE

On the recommendation of the Committee for organisation of Symposia the Council of the National Institute of Sciences of India at their meeting in May, 1959 decided to organise a Symposium on Thyro-gonad adrenal-pituitary relationships at New Delhi on October 2, 3 and 4 1959

A Steering Committee consisting of the following members was formed accordingly —

Dr B Mukerji *Lucknow* (Convenor)
Dr V R Khanolkar *Bombay*
Dr S Mitra *Calcutta*
Dr B B Dixit *New Delhi*
Dr A B Kar *Lucknow*
Dr P Bhattacharya *Izatnagar*

The Steering Committee secured the co-operation and help of those working in this field in different institutions both in this country and abroad As a result, abstracts of 27 papers were received three of which were from abroad It has however, not been possible to include all of them in the Bulletin as only 18 full papers were received from the authors for publication

The Symposium was inaugurated by Dr K N Bagchi in the absence of Prof

and to those who helped the Institute to make this Symposium a success

EDITOR

CONTENTS

Section D

Pituitary-Adrenocortical Mechanisms

OPENING REMARKS

by B Mukerji

the nervous and the endocrine systems

The role of nervous system in homeostasis has been the theme of brisk study and research since the beginning of the present century when Sherington's *magnum opus* on this subject was published As a result neuronal interrelationships and the function of the various integrative portions of the central nervous system have been clarified A fundamental concept of hormonal interrelationships, however, and their integrative relationships with the "milieu interieur" has been slower in evolving, most of the progress having been made only in the past two or three decades

latter either through this medium or through nervous pathways

As a regulator of endocrine activity, the anterior lobe of the pituitary occupies an outstanding position It stimulates the adrenal cortex (ACTH and perhaps growth hormone, 'adrenal weight factor'), the pancreas (diabetogenic hormone), the thyroid (thyrotrophic hormone) and the gonads (FSH, LH and LTH) In this manner it participates in almost all aspects of bodily functions

Thus, under the influence of ACTH and possibly growth hormone, the adrenal cortex is stimulated to produce glucocorticoids, perhaps mineralocorticoids and certain sex hormones particularly the androgens The corticoids influence carbohydrate metabolism, electrolyte metabolism, blood pressure, smooth muscle contraction, thymus and other lymphatic structures Theoretically some of the adrenocortical androgens should be able to maintain the male accessory genital organs in castrated animals but in practice this possibility has not been satisfactorily realised This is but one of the many unsolved

quite understood

The thyrotrophic hormone (thyrotrophin or TSH) acts on the basal metabolic rate, growth, differentiation and metabolism *via* the thyroid hormone (thyroxine) whose production it sitmulates The thyroid and the adrenal cortex have a close partnership perhaps to maintain the cellular energetics at the optimum level The thyroid hormone also has profound influence on pituitary-gonad mechanisms in several major directions (1) timed release of FSH and LH (2) peripheral passage of gonadotrophins to the gonads

(3) physiology of the gonads with especial reference to follicular maturation and estrogen-secretion by the ovary, and spermatogenesis and androgen secretion by the testis (4) physiology of the accessory sexual organs and particularly their sensitivity to homo-

The intricate and closely articulated nature of gonad-pituitary mechanisms can be best appreciated by a casual examination of the hormonal interrelations during the

FSH and LH production In addition, they stimulate luteotrophin (prolactin) and

mammary gland

'luteal phase' changes

lobe and inhibit FSH

terone.

The regular recurrence of the female sexual cycle perhaps may be explained by the periodic production of FSH with a resultant increased formation of estrogens, the latter in turn inhibit FSH and LH formation to a point where the ovary fails to form sufficient estrogens to maintain this inhibition so that FSH and LH production again rises, and the process is reinitiated

Some 20 years ago, animal experiments demonstrated that the organism responded

to widely different stimuli such as infections, intoxications,

r fatigue or x-irradiation. Each of these stressor

ny cases cally opposed The common

organ te of 'biologic stress'. The

ped m dless of the nature of the

ummati rimposition of the specific

provocative agent active p y 'n n-specific stress'.

actions 'stressor'

The prefactory nonspecific adaptive reaction the so-called 'alarm reaction' which characterizes body's response is in the nature of a call to arms of the organism's defences Detailed studies indicated that this was merely the initial phase of development of a more [illegible] alarm reac- [illegible] in which [illegible] ted adapta tion is lost Thus continued stress after adaptation leads to the 'stage of resistance' At this time inurement to the evocative stressor increases but resistance to other damaging agents diminishes When adaptation fails the 'stage of exhaustion' ensues with neutralization of all defence mechanisms and eventual death

The stressor such as trauma infection or burn acting on the targets evokes a stimulus which excites the anterior pituitary to produce ACTH under certain circumstances it may also cause a discharge of TSH growth hormone or even gonadotrophins The nature of this first mediator between the directly injured organ and the anterior pituitary is as yet unknown Corticotrophin stimulates the adrenal cortex to produce pre- [illegible] reactions in the target organs such as anabolism and augmentation of granuloma formation and of allergic responses, primarily stimulating the connective tissue Part of this action is undoubtedly not mediated through the adrenal cortex but this direct effect sensitizes the connective tissue elements to the essentially similar actions of the mineralo-corticoids In other words there is a peripheral synergism between growth hormone and mineralocorticoids However, by itself growth hormone cannot maintain the adrenal cortex in a functional state hence its 'corticotrophic effect is dependent on the simultaneous availability of ACTH It may be mentioned in passing that growth hormone has been considered by some to be associated with the so-called adrenal weight factor

The mineralo-corticoids and the gluco-corticoids have many opposing effects Deficiencies and imbalances in either the production or the activity of these corticoids result in derangements of the normal adaptative mechanism and may result in the production of certain maladies considered to be essentially diseases of adaptation [illegible] to stress Among these particular attention has been given to dietary protein which appears to increase the production and/or activity of growth hormone and to sodium which augments the effect of mineralocorticoids on certain target organs especially the kidney

[illegible] bolic derangements which abnormally alter the sensitivity of the target organs to growth hormone ACTH or corticoids and (4) although the pituitary adrenal system plays a prominent role in stress syndrome other organs which participate in the latter such as

the nervous system, liver or kidney, may also respond abnormally and become the cause of disease during adaptation to stress

I would like to emphasize here that vigorous and concerted research on thyro-gonad adrenal-pituitary mechanisms might help to solve one of our grave national problems

created where none would otherwise have existed, pregnancies have been saved ... would have terminated, diseases have been thwarted which would have proved fatal and finally the span of life has been significantly extended. In most respects the impact of endocrine research upon man's life has been similar to that provided by other areas of biological study, all of which taken together have in a relatively short time had a profound effect on world population If this trend in population increase is not checked, a serious situation resulting from food shortage is bound to arise in not too distant future The only effective manner by which a balance between food availability and population increase can be maintained is by evolving some method of controlling human fertility This does not appear to be an impossible task if the endocrine interrelations are better understood and some method adopted through which the extremely delicate mechanism of fertilisation of the ovum by spermatozoa can be attacked at some vulnerable point A study of sex gland endocrinology alone is

ties of human fertility control and can adopt physiologic or pharmacologic means to control it at will when necessary

Lastly I would like to elaborate a little more on the fact that the nervous and endocrine systems are integrated structurally and functionally in various ways and in various

which are elaborated and secreted by the neuroendocrine system, are members of a unitary constellation of metabolic activators and inhibitors that exhibit a phenomenal spectrum of individual effects and interrelated activities In the performance of its ubiquitous metabolic task, the neuroendocrine system, is interlocked functionally with the vascular system The vascular system supplies the neuroendocrine system with the raw materials required for synthesis of its internal secretions and it serves coincidentally as the body-wide distribution agency of the manifold hormones which it produces and secretes

precipitate functional or organic pathology of clinical consequence However, very little

has been discovered as yet about the psychobiologic mechanisms and pathways through which environmental stress especially that of a sociocultural nature, is perceived,

1) Hypogonadism in both sexes
2) Menstrual disorders of psychogenic origin
3) Exopthalmic goiter
4) Obesity
5) Cushing hyperadrenocorticalism
6) Psychoses of schizophrenic and epileptoid types
7) Hypertension
8) Diabetes of pancreatic pituitary and adrenocortical origin
9) Hypo and hyperpigmentation
10) Pituitary dwarfism and acromegaly
11) Cancer of various endocrine glands or their target organs like.
    (a) Ovarian and testicular tumors
    (b) Adrenal carcinoma with virilism in female
    (c) Pituitary and thyroid cancer
    (d) Breast and prostatic cancer

It will thus be realised that the field of neuroendocrinology is gaining in strength and

national welfare

SECTION A

THYRO - GONAD - PITUITARY MECHANISMS

THE NATURE OF INFLUENCE OF THE THYROID ON GONAD PITUITARY MECHANISMS

by AMIYA B KAR

Central Drug Research Institute, Lucknow

Hypo-or hyperthyroidism induced by pharmacologic means provoked various types of alterations in gonadal physiology Thus, persistent thiourea administration to

weight, the cholesterol concentration of the testis too registered a significant rise The latter tended to signify that the rate of genesis of androgen from cholesterol was reduced

treatment whereas, it may be recalled that 91 6 per cent of the animals not injected with

To return to gonad-pituitary system, the existence of a reciprocal relationship

to sensitize the adrenal cortex to endogenous ACTH released through the agency of the thyroid hormone (S N Roy, and Kar, 1958)

The histological picture of the testis of the hormone treated rats was in no way different from that of the controls, spermatogenesis continued vigorously and the Leydig cells did not show any evident atrophic changes

In contrast to the testis, ... TIT exerted interesting effects on the accessory genit ... was reduced significantly after T ... gard to the absolute weight but the decrease in relative weight was not significantly different from that of the controls although the trend of change was similar to that in the TIT group However, TIT exerted more drastic effects on the SV than T and this was evident from

of the SV of TIT treated animals revealed that the villi-like folds of this organ appeared

somewhat stunted and the secretory epithelium showed signs of atrophy at certain places resembling that of the castrates Nevertheless such atrophy of the epithelium was focal and not generalized as in the castrates the organ appeared active with innumerable granules in the epithelium and copious amounts of secretion in the lumen Repeated examination of sperms of the treated animals failed to show any particular abnormalities

In view of such unusual changes in the accessory genital organs the effects of thyroid hormones on fertility was investigated However as the influence of the two on the genital organs was qualitatively more or less similar the one (TIT) which produced more marked changes was used in these and the subsequent studies The results of this test showed that TIT had no effect on fertility of the recipient males the litter size and weight of the individual young remained unchanged (Kar *et al* 1959) The SV weight showed consistent reduction as before

These studies therefore indicated that the *nature* of effect of hyperthyroidism on the genital organs of young male rats was different from that in old rats In the former an induced hyperthyroid condition interfered with testicular activity probably through a disturbance of pituitary gonadotrophin output and the changes in the accessory genital organs were evidently secondary to an inhibition of androgen production by the atrophied Leydig cells In older rats on the other hand a similar hyperthyroid condition did not seem to disturb testicular function either gametokinetic or endocrinic although a direct inhibitory influence on the genital accessories was a consistent finding Curiously such effect on the accessories did not result in any commensurate reduction in fertility Nevertheless it was evident that the morphogenetic nature of testicular changes whenever they occurred were fundamentally similar in hypo- or hyperthyroidism This fact suggested that normal testicular activity was largely dependent on an optimal ratio of the level of thyroid hormone to that of GTH any imbalance of this ratio was detrimental to the functional status of the testis (S N Roy *et al* 1955) The reciprocal thyro-gonad pituitary mechanisms were adjusted suitably to maintain this optimal ratio between these hormones

Further studies were carried out to establish that the thyroid hormone did in fact exert such a direct influence on accessory genital organs (Kar *et al* 1959) It was noticed that the administration of TIT to castrated male rats caused a significant decline in absolute weight of the already infantile SV but the relative weight did not reveal a significant change TP alone injected to castrates daily for 12 days caused the expected stimulation of the SV weight But when TP was given conjointly with the thyroid hormone beginning from the seventh day of TIT administration the characteristic increase in SV weight was less in comparison to the group injected with TP alone However this difference was statistically significant only with regard to the absolute weight but when the relative weight of the organ was considered the difference was not statistically significant In contrast prostatic acid phosphatase activity tended to show noteworthy alternations Thus castration caused a significant decline (41 8 per cent) in enzyme activity from the normal controls but administration of TIT evoked as much as 72 1 per cent inhibition Phosphatase activity in the castrates given TIT was significantly (52 per cent) less than that in the untreated castrates TP therapy restored the enzyme activity to normal level but when TP was injected along with TIT phosphatase activity tended to be less though not in a significant manner On percentage basis prostatic acid phosphatase activity in the group treated with TP alone was 67 7 per cent higher than in the untreated castrates but in the group simultaneously injected with TP and TIT the enzyme activity was only 21 7 per cent higher In this connection our unpublished observations indicated that the duration of action of TP too was influenced by TIT

Studies on influence of TIT on androgen action led us to explore whether the thyroid hormones had any comparable effect on gonadotrophins at the periphery (Kar *et al* 1954) Accordingly, prepuberal female rats were injected with PMS gonadotrophin twice daily for 10 days Another group received thyroxine daily in addition to gonadotrophic hormone at the same dose and for the same period It was noticed that the ovarian weight was significantly increase
in combination with thyroxine
of the ovary was significantly lo
Similarly, the uterine weight was stimulated in gonadotrophin and gonadotrophin plus thyroxine groups and there was virtually no difference between them in this respect. Cholesterol concentration of the ovary declined significantly after gonadotrophin administration but the difference between the two gonadotrophin groups was insignificant. Histological examination of the ovary of control animals presented a typical infantile picture with ovocytes and immature follicles in various stages of growth and atresia The beginning of antrum formation was seen in only occasional follicles Thecal
fibrocellular
hin treated
formation
s were rare

Apart from these there was hypertrophy of the interstial epitheloid elements but the interstitium as a whole was considerably obliterated owing to the enlargement of the follicles and bulky luteinization The entire organ was highly vascularized The gross histologic appearance of the ovary of GTH plus thyroxine treated rats was also indicative of a stimulatory response However certain details were noteworthy Thus healthy mature follicles and corpora lutea were common as in gonadotrophin treated animals

These findings suggested that thyroxine tended to reduce some of the untoward consequences of excessive and precocious excitation of the immature ovary by gonadotrophic hormone Thus the tendency of gonadotrophin to stimulate all of the available normal follicles to maturity irrespective of their extent of differentiation (and thereby leading to artresia hemmorhage and cyst formation) was curbed by thyroxine However this effect of thyroxine was not directed against the normal physiologic action of gonadotrophin on the ovary as follicular maturation ovulation luteinization and evokation of endocrine activity as shown by uterine changes were consistently observed Only the pathological effects of untimely and excessive ovarian stimulation by gonadotrophic hormone were minimized It was possible that thyroxine either acted at the level of the ovary and caused slower utilization of gonadotrophin or more likely thyroxine permitted the sojourn of only physiologic amounts of gonadotrophin from circulation to gonads and the excess was inactivated may be by stimulation of anti-hormone formation

tantiated by further studies it will be an important peripheral mechanism in the maintenance of gonad-pituitary homeostasis Incidentally, we are also exploring the possibility of thyroid participation in the mechanism responsible for the formation of anti-gonadotrophin anti-bodies or anti-hormones

Coming to the reciprocal influence of sex hormones on the thyroid mention may be made of one of our studies concerned with the effect of TP on thyroid is in doses known to evoke a differential response in the testis It is now a commonplace that low

trophin output

cularity was conspicuous and even some engorgement of the vessels was noticeable

In order to determine

a stimulation of pituitary

the low dose of TP plus

devoid of vacuoles The vascularity of the organ was poor In the T plus TP treated

S. N Roy *et al*, 1956)

We also examined the testis of these animals and obtained interesting informations (Roy *et al*, 1955) Thus T alone caused a loss of testis weight and histological indications

rarely beyond the primary spermatocyte stage and there was profound disorganization and sloughing of the seminiferous elements The adverse effects, therefore, seemed

but not in a statistically significant manner; the histological appearance too was normal Cholesterol concentration was similar as in the controls

These facts suggested that (1) the low dose of TP stimulated the thyroid through

hand, T *per se* tended

to make the condi-

ised both TSH and

gonadotrophin output of the pituitary as shown by the stimulation of both the thyroid and the testis However, depression of TSH production by T did not dampen the capacity of TP to excite pituitary gonadotropnin release. Even T with all its intrinsic ability to suppress pituitary gonadotrophin activity was evidently ineffective against such powerful stimulus provided by TP for outpouring of pituitary gonadotrophin. In other words, these studies indicated that at the primary level at least the differential action of the two doses of TP on gonadotrophin output was independent of their influence on TSH release.

In the panoroma of endocrine inter-relationships which we are privileged to witness in this symposium the thyro-gonad-pituitary mechanisms occupy an outstanding position The true nature of relationship between these three endocrine glands is yet to be

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EFFECT OF MILD HYPERTHYROIDISM ON THE REPRODUCTIVE PERFORMANCE IN MALE AND FEMALE RATS UNDER CLIMATIC STRESS (HEAT)

by D N Mullick

Animal Nutrition Division, Indian Veterinary Research Institute, Izatnagar, U P

Abstract

The results obtained in the present experiment indicate that summer months depress sexual performance or fertility in adult male and female rats

There was a significant difference in the weight of the testis and the diameter of the follicles of testis in summer and winter months Injections of different doses of thyroxine solutions have considerable positive effect in the activity of the testes as studied by the weight and the histological appearance

In female rats at higher atmospheric temperature the decrease in the number of estrous cycles and their longer duration and increase in the gestation period were recorded

These conditions can be improved by supplementing critical amount of thyroprotein in the diet

Considering the lower reproductive efficiency of the animals in the tropics, the present investigation was started first to study the effect of natural fluctuation of the atmospheric conditions with the activity of the reproductive capacity of the male and female rats and their reactions with the thyroidal supplement during thermal stress

Experimental

Eight male normal adult rats of the Institute stock of known performance were killed when they attained age of 180 days in winter months when the atmospheric temperature was 60°F In summer months (atmospheric temperature, 100°F) 48 male rats of the same strain and about 160-165 days old were divided into 6 groups One group was fed with Institute stock diet which was served as control The other five groups were supplemented by 0 1 per cent thiouracil (BDH) and simultaneously different doses of thyroxine solution (BDH) ranging from 3 to 7 μgm per 100 gm of live weight were injected daily for 15 days The dose of thyroxine solution to be injected was calculated from the daily body weight of the animals Food and water were provided *ad lib* The testes of all the groups in summer and winter months were removed and weighed in a chemical balance corrected up to tenth of a milligram They were fixed in susa solution and paraffin cut sections were examined histollogically after staining with hematoxylin and eosin

The statistical analysis of the data has been made according to the methods given by Snedecor (1946)

Twelve adult female albino rats of the Institute strain were fed with stock diet in winter months when the room temperature was 60°F Similarly, 24 rats were fed on stock diet in summer months when the room temperature was 90°F Vaginal smears were taken daily in both these groups during a period of 4 weeks The selected rats have littered at least once

In the summer experiment, after 4 weeks the animals were divided into two groups, one of which has continued on the stock diet and the other fed with the stock

diet supplemented with a proprietary preparation of 0 02 per cent thyroprotein ("Protamone") During the period of observation the vaginal smears were obtained daily on a slide, dried in an incubator, stained with Giemsa stain and examined under the microscope Evans and Long (1921) classification of the oestrous was followed At least 6 oestrus cycles were recorded before the animals were allowed to mate. Successful matings, the gestation period, and the number and weight of the litters were recorded The litters were weighed in a group within 6—12 hr after birth

Results

The average weights of the testes with the standard error were 1007±18 6 and 773±22 6 mg per 100 gm of the body weight of the rat at the temperatures of 60°F and 100°F (Table 1) The weight decreased with the increase in the air temperature The difference in the mean values between the seasons was highly significant according to Fisher's 't' test In the summer month when the activity of the organ was minimum, the animals were injected daily with different doses of thyroxine ranging from 3 to 7 μgm per 100 gm body weight of the rats The weights of the testis gradually increased with the quantity of thyroxine injected and the values were examined statistically according to analysis of variance to find out whether the differences in the weights of the organs due to treatments were significant or not It was found that the F value between the treatments and weights of the organs was highly significant at 1 per cent level

Table 1

Showing the average values of the weight or testes and the diameter of the testicular follicles at 60°F and 100°F and at different doses of thyroxine injected

Air temperature °F			Weight of Testis (mg) Treatment with thyroxine μgm				
60	100	t	3	4	5	6	7
M ±S E	M ±S E		M ±S E	M ±S E	M ±S E	M ±S E	M ±S E
1007±18 6	773±22 6	**	975±20 3	810±19 8	850±26 9	892±21 4	928±30 1
			Diameter of the testicular follicles (Microns)				
248±6 9	302±5 45	**	344 6±6 98	320 6±8 75	306 0±7 58	283 8±9 69	272.4±8 12

** Highly significant at 1% level

The histological examination of the testes of rats in winter months showed increased spermatogenic activity when compared with that in summer months (Pl 1) The primary spermatocytes and spermatids were more prominent and showed marked mitotic activity Most of the lumina of the tubules contained numerous mature sperm, and the spermatogonia showed active proliferation and the cells were well organised At temperature 100°F testes of rats showed decreased spermatogenic activity with atrophic and degenerative changes in some of the seminiferous tubules and cellular disorganisation The cells were very few With the injection of thyroxine the picture gradually changed and the activity could be brought to one like the winter stage especially in the two groups receiving 6 and 7 μgm of thyroxine which showed increased spermatogenic activity The

The effect of the different doses of thyroxine on the diameter of the follicles subjected for their significance test by analysis of variance It was found that size dif significantly at 1 per cent level between the different doses of thyroxine

The average number of oestrous cycles and the duration of each cycle in experimental group are presented in Table 2 The mean differences between the v for the summer and winter groups and between the thyroprotein treated and untr groups and untreated groups in summer are highly significant according to Fisher' test

TABLE 2

Showing the reproductive performance of the rats

Room Temperature	60°F	90°F	90'
		Treated with 0 02 °(	
Number of animals	12	12	1.
Average number of cycles	8 7±0 34	6 1±1 08	7 9±
Interval of each cycle days	4 50±0 34	4 96±0 51	4 80±
Gestation period, days	19 6±3 1	23 0±4 1	20 9±
Successful mating, percent	72	66	7(
Average number of young/litter	8 0±1 92	7 6±1 04	7 7±
Average weight of young	5 1±0 5	4 5±0.4	4 9±

DISCUSSION

The experiments, reported here, demonstrate rather clearly that higher atmos temperature has pronounced influence in the reproductive performances in male The level of injected thyroxine also influences the development and improvement sex organs according to the activity of the testes as judged by their size and histology

Dempsey and Astwood (1943) and Mullick (1959) showed a decrease in the t xine secretion rate at higher temperature in rats Presumbly, this is one of the ho thermic mechanisms that permits the animal to compensate for heat stress The anim

than good to improve the condition of the animals, but the use of physiological may turn out to be beneficial rather than injurious to the health of the animals physiological dose should be worked out before actually any application in th therapy starts

The mechanisms whereby the thyroid influences sexual function have not been

gonadotropic hormone content of the pituitary as a result of the thyroidectomy his

Histological Sections of testis of normal adult rat under different atmospheric temperature and treatment with thyroxine



A —Testis Section of rat at 100 F showing limited spermatogenesis and degenerative changes in the tubules



B —Improvement of all the above conditions by supplementing with 4 μgm thyroxine per 100 gm body weight



C —Testis section of rat at 60 F showing numerous mature sperm and spermatogenic cells showing activity with greater vascular regions

reported in rabbits (Van Dyke and Cheu, 1942) in rats (Stein and Lisle, 1942) and in goats (Reineke *et al*, 1941) Little information is available to indicate whether mild hyperthyroidism would increase the output of gonadotropins by the pituitary The administration of thyroid to rabbits resulted in a lowered content of pituitary F S H and increase in L H (Chu and You 1954)

The present results may also be explained in part by a modifying effect of the thyroid hormone level on the response on sex organs to gonadotropins, since the response of the testes is increased by mild hyperthyroidism and reduced by hypothyroidism (Meites and Chandrashekar, 1948) Furthermore the response of the accessary testasterone is increased by thyroxine (Masson 1947)

The supplementation of thyroprotein in summer months (Maqsood and Reineke 1950) to improve the general condition of the reproductive performances in males may be due to the well established role of this endocrine in controlling the general metabolism in the body which may simultaneously help to increase the consumption of food Thus the nutrients and the higher metabolism may be chief factors in the improvement of sexual condition of the animals in supplemented group

The results of the present experiments support the view that hypothyroidism due to higher environmental temperature interferes with normal reproductive function in female albino rats also The decrease in the number of oestrous cycles and their longer duration in summer may be due to the effect of either lack of stimulus of the general metabolic activity as a result of hypothyroidism or due to slowing down of the secretory activity of the pituitary or of both these factors on the function of the ovary The increase in the gestation period during summer observed in the present experiments is in conformity with the observations of Krohn and White (1950) on hypothyroid rats but is much less pronounced than in the case of Ukita's (1919) findings in rabbits

The improvement in the reproductive performance of female rats during summer on supplementation of the diet with thyroprotein may be related to the well-established role of this hormone in stimulating the general metabolism of the body and the increased consumption of food which may result therefrom Thus the increased intake of nutrients and the higher metabolic rate may be the chief factors in the improvement of the reproductive performance of the animals in the group receiving the supplemented diet

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SUITABILITY OF I^{131} (RADIO-IODINE) IN EVALUATION OF THYR FUNCTION IN THYROID-PITUITARY-GONAD INTER-RELATION

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and

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Abstract

By I^{131} uptake in the Thyroid gland percentage excretion of I^{131} in Urine and bound Iodine (PBI^{131}) in blood (indicating thyroxine or such compound) have all been quanti measured

Since the introduction of radio-iodine (I^{131}) the function of thyroid glan role of T S H (thyrotrophic hormone of anterior pituitary), and influence of the interfering with the action of T S H on synthesis of thyroxine, have been subj re-investigation in various laboratories (Hertz *et al*, 1938, Hamilton, 1938, Hertz,

Already, a voluminous literature (Freyers 1956, Ansell *et al*, 1953, Storaasli, Myant and Pochin 1949 Berson *et al*, 1952, Myant, 1952, McConahey and 1956, Pochin ... Howard 1958, Shelne ... or suit of different ... on of th gland These parameters are directed mainly to assess (1) the rate of up-take of iodine by the gland (2 hours to 48 hours) (2) the rate of release of I^{131} labelled th hormone, and (3) rate of clearance of I^{131} from thyroid gland

But iodide in thyroid space" (iodine trapping by thyroid gland and subs ... "iodide ... yroidal ... roid spa

In the last few years (Fraser *et al*, 1953, Goodwin *et al*, 1951, Rotblatt and M 1953 Paley *et al* 1955) importance has been attached to (1) rate and percentage of exc

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of I^{131} in the urine and (2) rate and percentage of release of different fractions of thyronine and (3) capacity of thyroid to trap and/or bind iodine Influence of exogenous T S H , on these is being studied extensively in health and disease by German, Swedish and other European workers (Brown-Grant, 1957, Stürm, 1959) and these data are being analysed along with experimental data obtained by hypophysectomy

However, anterior pituitary gland releases many hormones, so it is rather difficult to believe that the results obtained from hypophysectomy will be comparable to those obtained by T S H administration or inhibition of T S H But a careful search in literature revealed that disorders in thyroid gland were envisaged, when patients reported for treatment of disturbances of growth or metabolism e g , cholesterol, or pigment metabolism, diabetes, cushing syndrome etc Available methods were not enough to study thyroid function

In recent papers, monographs, and publications of colloquia or symposia, results of long term clinical use of different tests for studying thyroid-function have been published and different methods have been compared The general impression from such papers is that there is little to choose between any of the similar tests as regards extreme precision (Fraser 1956, Oliver and Ellis, 1957, Chamman and Maloof, 1955) But if the tracer test result is compared with patient's clinical condition, in 80-90 per cent cases, interpretation is correct and in doubtful cases, further enquiry reveals valuable data which would have been ignored

So in our unit for the last three years I^{131} has been used in assessing the function of thyroid gland in cases reporting to out-patient's department or admitted in-doors with diagnosis of primary or secondary or doubtful thyroid disorders, but associated with some clinical manifestations of disturbances in growth or metabolism We have also attempted to assess the thyroid function during pregnancy (full term) before the expected date of onset of labour so that we can measure the radio-active iodine accummulated in the thyroid of the new born child - which may throw some light relating to transgress of maternal hormone across the placenta in the foetus

The purpose of this paper is to apprise you of these data which will reveal clearly that the thyroid gland is intimately linked with almost all the hormones of the anterior pituitary

Methods and Materials

The subjects of the present series did not have any treatment with iodine compounds thyroid extract, antithyroid compounds, inhibiting drugs before the investigations were carried out, nor any X-Ray examination was carried out previously with contrast medium

Percentage uptake and Thyroid/Thigh Ratio

For the purpose of study of the absolute quantity of I^{131} (expressed as percentage of the administered dose) taken up by the thyroid glands, radioiodine in the form of sodium iodide* having activity between 30-50 μC (in adults) is administered orally, on empty stomachs Counts over the thyroid glands are made at selected intervals with the help of scintillation counter and counting set No PW4032 At the time of each count, care is taken that the geometrical relationship between the counting set and the

* Radio-iodine was obtained in the Carrier free state from M/s N V Philips Roxane Pharma-Chem Industries Isotope Laboratory DUPHAR

subject is kept constant As the radioactive decay occurs in radial directions, the ab disintegration of the isotope can not be measured Therefore we measure a co

activity in the neck and are deducted together with the background from th values to come to the absolute thyroidal uptake expressed as counts per minute over the area

In order to convert the thyroid count as percentage of the dose administered model of thyroid of approximately normal shape and size, is used in which identical to that administered orally is instilled This model is known as phantom count over the phantom kept at the same constant geometry as observed in the c individual subjects and using the same counting set is taken From these two percentage uptake of I^{131} by the thyroid at any desired interval can be calculated second hour thyroid and thigh readings corrected as above the thyroid/thigh r determined

Urinary Excretion (Percent)

Before administration of I^{131} the subject is made to empty his bladder Aft dose of I^{131} is administered urine during the next 24 hours is collected The volu urine is measured Sample pan containing 0 1 ml of urine is prepared and drie counted in a G M counter The total activity excreted in the 24 hours urine is esti as cpm

het co inted dmini rine is

expressed as the percentage of dose administered

Protein Bound radio-Iodine (PBI^{131})

The precipitate is washed with trichloroacetic acid several times in order to eliminat inorganic iodine The precipitate is then dissolved in (2N)NaOH and the whole qu is transferred to a glass planchet and dried A standard of activity 002-005 μC prepared at the same time and dried Counts of the PBI^{131} sample and the standa taken in the same counting set under the same geometrical set up The plasma p bound activity is then calculated and expressed as percentage of the dose per lit plasma

of contamination minimised

In all estimations whether in vivo over the neck and thigh or in the case of plan the backgrounds of scintillation and G M counters are recorded and considered.

Results

The results of investigations in normal conditions and in different disorders of thyroid gland - diagnosed clinically are shewn in Tables I & II

TABLE I

	TEST	Hypo-Function (myxoedema)etc	Normal range	Hyper function
1	Four hours thyroid uptake	<10%	10-40%	>40%
2	Twenty four hours thyroid uptake	<20%	20-50%	>50%
3	Two hours thyroid thigh	<2	2 10	>10
4	Twenty four hours urine excretion	>50%	40-70%	<40%
5	Forty eight hours Plasma P B I 131	—	0 1%—0.2% —0 4% per lit	>0.4% per lit

Discussions

Compared with data in normals the analysis of Figures in Table II permit us to arrange disorders in Thyroid functions (diagnosed clinically) with relation to Radio-Iodine test as follows

1 *Second hour up-take*

(a) Very poor in Cretins
(b) Poor in hypothyroidism
(c) High in pregnancy (higher limit of normal or little above it)
(d) Very high in hyperthyroidism (thyrotoxicosis)

2 *Fourth hour up-take*

(a) Low or lower limit of normal range in myxoedema hypothyroidism with or without diabetes
(b) Lower limit of normal in cretins
(c) Very high in hyperthyroidism

Twentyfourth hour up-take

(a) Very low in cretins hypothyroidism with or without diabetes
(b) High above maximum limit of normal range in hyperthyroidism

Percentage excret on of I131 in Urine up to twentyfourth hour

(a) Very low in hyperthyroidism (thyrotoxic)
(b) Low in hyperthyroidism and mongolism
(c) High in hypothyroidism
(d) Very high in myxoedema

TABLE II

Case No	Age	Sex	Thyroid/ThighRatio at the *Second hour* (2-10)	Percentage uptake of I^{131} by Thyroid Gland at 4th hour (10-40)	Percentage uptake of I^{131} by Thyroid Gland at *24th hour* (20-50)	Percentage excretion of I^{131} in urine upto *24th hour* (40-70)	Protein bound Iodine (PBI^{131}) at the 48th hour (0.1-0.4)	REMARKS: *Clinically referred*	REMARKS: Laboratory Diagnosis
1	22	M	6.55	18.85	29.08	60.59	0.218	Normal	Normal
2	3	F	0.7585	12.5	2.07	not collected	0.0434	Cretin	Hypothyroidism
3	12	F	6.77	19.63	33.09	27.22	0.29	Mongolism	Normal
4	7	M	3.87	12.20	25.14	33.14	1.12	Goitre	Hyperthyroidism
5	16	M	4.0	23.30	45.44	38.45	0.7308	Gigantism	Hyperthyroidism
6	41	F	8.47	23.76	45.5	38.00	0.32	Acromegaly	Normal
7	25	M	2.24	8.33	4.65	47.37	no activity	Myxoedema	Hypothyroidism
8	29	M	1.73	10.26	17.22	77.95	0.054	*Anxiety state* Trembling Palpitation	Hypothyroidism
9	47	M	20.84	20.23	36.82	20.76	0.0023	?? Cushing ?? Myxoedema	*Thyroxine deficiency*
10	40	F	94.5	74.5	68.25	9.44	0.547	Thyrotoxicosis	Hyperthyroidism
11	28	M	45.6	56.39	71.63	30.20	0.515	Thyrotoxicosis	Hyperthyroidism
12	46	M	10.91	16.86	36.02	42.71	0.229	Exophthalmos (? Hyperthyroidism)	Normal (Cortisone-receded)
13	48	M	8.78	12.5	27.38	52.22	0.269	Under investigation (?Effort Angina)	Normal

TABLE II (*Contd*)

Case No	Age & Sex		Thyroid/ Thigh Ratio at the Second hour (2-10)	Percentage uptake of I^{131} by Thyroid Gland, at 4th hour (10-40)	24th hour (20-50)	Percentage excretion of I^{131} in urine upto 24th hour (40-70)	Protein bound Iodine (PBI^{131}) at the 48th *hour* (0 1-0 4)	REMARKS: Clinically referred	REMARKS: Laboratory Diagnosis
14	28	F	105 46	79 07	91 81	8 45	0 5619	Congestive Cardiac failure with (*Thyrotoxicosis?*)	Thyrotoxicosis
15	37	F	6 22	18 52	39 42	39 96	0 1617	Congestive Cardiac failure with (Myxoedema?)	Normal
16	63	M	9 95	18 29	44 30	35 97	0 459	Diabetes	Hyperthyroid
17	52	M	1 83	4 59	4 16	56 03	no activity	Diabetes	Hypothyroid
18	40	F	8 15	14 15	39 35	39 70	0 232	Normal	Normal
19*	24	F	13 99					Pregnancy (Full term)	Normal
20*	22	F	27 2					Pregnancy (Full term)	Slightly above Normal
21*	Child (New-born)				(N) 21 9			New born	Normal
22(A*)	16	M	37 17					Gigantism	Hyperthyroidism
22(B*)	18	M	6 83					Normal	Normal
22(C*)	13	M	4 3					Normal	Normal

* Indicates Intravenous administration

THYROID AND PHYSIOLOGY OF ACCESSORY SEX ORGANS

by J S Rawat and A Roy

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The hormones secreted by the various endocrine glands are directly or indirectly involved in the normal functioning of the reproductive processes in both the sexes, and the thyroid hormone appears to be one of those which is intricately linked with reproduction In the present communication is reported the effects of hypothyroidism on the development of male reproductive organs and the composition of the seminal vesicles

ted

The seminal vesicles were dissected out alongwith their contents, weighed and macerated with sand in a glass mortar containing trichloracetic acid Fructose was estimated in the extract The results are given in Tables 1 to 8

Progress of hypothyroidism as a result of thiourea administration resulted in a decrease in weight of the seminal vesicles and statistically significant from 14th day

With advancement of age, the seminal vesicles increased in size and weight inspite

vesicles was found to be very much higher and was nearly the same as those which were :reated with thyroxine and gonadotrophin alongwith thiourea (Table 3)

The effect of the plane of nutrition is also shown by the changes in the weight of the :stis The weights of the testis of hypothyroid and pair fed control rats were markedly wer than the weight of the testis of animals fed ad lib (Table 3) It was also noted that hough the simultaneous administration of gonadotrophin alongwith thyroxine and ourea increased the weight of testis, it was still significantly lower than those of un-ated animals fed ad lib

It was further observed that as hypothyroidism establishes itself, not only the weight the fructose concentration in seminal vesicles decreases markedly indicating thereby decreased testicular hormone elaboration (Table 8) Administration of thyroxine along-with thiourea checks the derangement It is also evident that the seminal vesicles could not get back to normal size and function when thiourea feeding was continued for 42 days and then stopped The size of the seminal vesicles as well as the fructose content failed to return to normal during the observed post thiourea feeding period Chronic hypothyroidism, therefore, appears to produce a more permanent damage to the repro-ductive organs

It appears that the alteration in the weight and function of the male reproductive organs during hypothyroidism is to a significant extent due to the lower caloric intake and the main effect of undernourishment is on the elaboration of the gonadotrophic hormones by pituitary This is substantiated by the observation that when animals of same weight are allowed to eat ad lib the size of the seminal vesicles as well as the various constituents it contains or produces are as high as those which received besides thiourea and thyroxine, gonadotrophin also (Table 3)

TABLE 1

Weight in mg of seminal vesicles and testis during the progress of hypothyroidism

		DAYS				
		7	14	21	28	42
Seminal Vesicles	TU Fed	95 ± 21	76 ± 20	89 ± 21	110 ± 20	214 ± 21
	PFC.	115 ± 22	104 ± 24	111 ± 25	223 ± 20	365 ± 27
Testis	TU Fed	1620 ± 61	1340 ± 35	1800 ± 61	1450 ± 30	1980 ± 31
	PFC	1480 ± 45	1180 ± 41	1640 ± 55	1200 ± 40	1820 ± 34

TABLE 2

Weight in mg of seminal vesicles and testis during the regress of hypothyroidism following withdra[wal of] thiourea after being fed for 28 and 42 days

		After 28 days DAYS				After 42 days DAYS	
		7	14	21	28	7	14
Seminal Vesicles	TU Fed	172 ± 25	306 ± 26	417 ± 28	421 ± 31	249 ± 18	300 ± 21
	PFC	236 ± 27	401 ± 35	434 ± 30	410 ± 29	336 ± 22	398 ± 27
Testis	TU Fed	1660 ± 40	1830 ± 50	1940 ± 80	2020 ± 40	2000 ± 31	2010 ± 31
	PFC	1560 ± 50	1980 ± 30	2040 ± 70	2110 ± 40	2150 ± 32	2100 ± 34

TABLE 3

Weight in mg of seminal vesicles and testis as affected by simultaneous administration of Thiourea and Hor[mones] continuously for 28 days Figures under the last column show weight of glands of animals fed ad lib

(For ready reference weight of these glands from 28 day thiourea fed animals and their p[air fed] controls have also been incorporated in the table)

	Pair Fed control	Thiourea	Thiourea thyroxine	Thiourea ACTH	Thiourea TX,G	Fed a[d lib]
Seminal Vesicles	223 ± 20	110 ± 21	250 ± 32	121 ± 31	850 ± 125	
Testis	1200 ± 40	1450 ± 30	1350 ± 44	1350 ± 45	1760 ± 61	2

TABLE 4

Fructose content of seminal vesicles of animals during the progress of hypothyroidism

DAYS	Group	Absolute content in mg	Per 100 gm of S V in
7	TU Fed	Trace	Trace
	PFC	Trace	Trace
14	TU Fed	Trace	Trace
	PFC	Trace	Trace

TABLE 4 (Concld)

DAYS	Group	Absolute content in mg	Per 100 gm of S V in mg
21	TU Fed	Trace	Trace
	PFC	122 ± 014	57 ± 70
28	TU Fed	Trace	Trace
	PFC	104 ± 035	47 ± 150
42	TU Fed	Trace	Trace
	PFC	158 ± 016	43 ± 50

TABLE 5

Fructose content of seminal vesicles of animals during regress of hypothyroidism maintained for 4 weeks

DAYS	Group	Absolute content in mg	Per 100 gm of S V in mg
7	TU Fed	069 ± 011	40 ± 60
	PFC	094 ± 013	40 ± 60
14	TU Fed	154 ± 021	50 ± 70
	PFC	100 ± 022	47 ± 50
21	TU Fed	181 ± .022	44 ± 50
	PFC	194 ± 020	45 ± 50
28	TU Fed	188 ± 031	45 ± 7.0
	PFC	190 ± .023	45 ± 60

TABLE 6

Fructose content of seminal vesicles of animals during regress of hypothyroidism maintained for 6 weeks

DAYS	Group	Absolute content in mg	Per 100 gm of S V in mg
7	TU Fed	Trace	Trace
	PFC	145 ± 042	43 ± 13.0
14	TU Fed	066 ± 010	22 ± 4 0
	PFC	144 ± 020	38 ± 5 0
21	TU Fed	149 ± 053	41 ± 15 0
	PFC	.235 ± 084	40 ± 14 0

TABLE 7

Fructose content of seminal vesicles as affected by simultaneous administration of Thiourea and Hormones continuously for 28 days Figures under last column show fructose content of seminal vesicle of animals fed ad l b

(For ready reference the composition of these glands of 28 day thiourea fed animals and their pair fed controls have also been incorporated in the table)

	PFC	Absolute Content in mg Thiourea	Thiourea Tx	Thiourea ACTH	Thiourea TX ACTH	Thiourea TX G	Fed ad lib
Fructose	1042 ± 0350	Trace	1122 ± 0250	Trace		5510 ± 0450	6120 ± 0612

TABLE 8

Summarise the results on the weight and fructose content of seminal vesicles during progress and regress of hyporthyroidism

		Weight of seminal vesicles	Fructose
During progress of Hypothyroidism	7th day		
	14th day	×	
	21st day	×	×××
	28th day	×××	×××
	42nd day	×××	×××
During regress of Hypothyroidism (A) Maintained for 4 weeks	7 days	×××	×××
	14 days		
	21 days		
	28 days		
(B) Maintained for 6 weeks	7 days	×××	×××
	14 days	×××	×××
	21 days	×××	×××

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DISCUSSION

Shah Dr Kar do you think that the adverse effect of thyroid hormones on the testis of young puberal rats are direct or secondary to a primary effect on the pituitary?

Kar This point is engaging our attention We are also studying the FSH and LH content of the pituitary of such rats injected with thyroxine and 3 5 3' triiodothyronine I think the effect of these hormones on the testis are largely pituitary mediated although the possibility of some direct effect cannot be entirely ignored. You may recall Eartly and Leblond s work in this connection who noted some intensification of hypophysectomy effects on the rats testis by thyroxine

Prasad [illegible] 3 5 3 triiodothyronine on [illegible] somewhat similar observations on guineapigs in R K Meyer s laboratory at [illegible] were able to show that LH had a direct effect on guineapig seminal vesicles

Kar Have you published your results Dr Prasad? This is indeed an interesting finding because to my knowledge prolactin is the only gonadotrophic factor which has been implicated by some to have a direct trophic effect on a male accessory sexual organ and that is the prostate.

Prasad These results are pending publication in *Endocrinology*

Kar *Dr Mukherjee your use of 30-40 c of I^{131} as a tracer dose surprised me particularly* when you are using scintillation counters This is in my opinion a rather high dose for tracer purposes. Would you like to comment on this point?

S R Mukherjee I agree with you Dr Kar that this is a rather high dose In collaboration with the Institute of Nuclear Physics Calcutta we are trying to find out the minimum possible dose of I^{131} which could be used for tracer purposes

Kar Could you tell me Dr Mukherjee whether your hyperthyroid patients with diabetes were insulin resistant or insulin responsive ? I have a feeling that diabetes in such patients were of the corticoid type due to attendent hyperadrenocorticalism and as such they were resistant to insulin

S R Mukherjee Your diagnosis is probably correct Dr Kar The hyperthyroid diabets were resistant to insulin

Ahuja I fully agree with Dr Mukherjee about the absolute necessity of diagnosing the nature of thyroid disorders first before resorting to therapy

Chairman Mukerji *Dr Mukherjee s comprehensive studies have emphasised once again* what a valuable tool the radio-isotope technique has been in medicine

SECTION B

ADRENOCORTICAL — GONAD — PITUITARY MECHANISMS

INFLUENCE OF CASTRATION UPON THE ADRENAL RESPONSIVENESS OF HYPOPHYSECTOMISED MALE RATS GIVEN CORTICOTROPHIN

by J N Karkun[*] and F W Landgrebe

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Abstract

Adult male rats weighing between 120-160 g were castrated and hypophysectomised 21 days [illegible]

The gonadal influence on the size and function of the adrenal gland is well documented In most mammalian species orchidectomy is followed by an increase in the size of the adrenal The process can be reversed by replacement therapy with androgen (Chester Jones 1957) Testosterone propionate is found to influence the adrenal [illegible]

[illegible] with this end in view

Materials and Methods

Adult rats having body weights ranging from 120 to 160 g were used for this experiment They were divided into two groups and kept under uniform husbandry conditions throughout the experimental period The first group was castrated through bilateral incision into the scrotum while the second group was sham operated and served as the control

After 21 days both the groups were hypophysectomised and the adrenal responsiveness was determined by measuring the depletion of adrenal ascorbic acid following the administration of 0 5 mg ACTH (Acthar Corticotrophin Armour) in 0 2 cc saline per rat into the animals The method of hypophysectomy the mode of administration of the hormone and the method of determination of adrenal ascorbic acid were the same as described by Karkun *et al* (1953)

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[*]Colombo Plan Trainee from Central Drug Research Institute Lucknow India

Results

Table 1 embodies the data regarding the change in adrenal ascorbic acid foll ACTH treatment into the two groups of animals The body weight of the ar the weight of their left adrenals at the time of sacrifice are also included therein

TABLE 1

Effect of Castration upon the Change in Adrenal Ascorbic Acid following Treatment with ACT

Group of Animals	Treatment	Body Weight of Animals G ±S E	L. Adrenal Wt Mg ±S E	Depletion of Ascorbic A 100G ±
I	Castrated	133 5±16 45	12 7±1 95	180±1.
II	Normal	129 0±18 11	11 6±1 34	134 3±1.

ϕ Depletion significantly greater than that in the control, t=2 3, 0 5>p>0 2

Discussion

hormone is supported by the finding of Gompertz (1958) who observed that treatm castrated rat with methyl testosterone reduced the sensitiveness of the adrenal to e nous ACTH In this connection it is interesting to note the findings of Carter (1956) administered oestrogen to hypophysectomised rats and observed that the sex hor produces an increase in the sensitivity of the adrenal to exogenous ACTH As known, oestrogen and androgen are often found to possess opposing physiological tions and this property is made use of in their therapeutic applications

Our conclusion, based on the present experimental results is opposed to advanced by Rennels *et al* (1953) In view, however, of the limited data at our di and also our different approach to the problem, it is difficult to account fo discrepancy It may, however, be pointed out that Rennels *et al* (1953) used ac weight changes as parameter of the tissue function, while we have used a bioche change in the adrenal gland, viz adrenal-ascorbic acid change for the same purpose criterion used by us is considered to more sensitive than that employed by the fo group of investigators (Sayers *et al* 1948)

The result of the present experiment thus indicates that testosterone b

organ under the influence of a particular hormone is well understood

References

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(2) *Syndromes in adults*—When the adrenal cortex products severe derrangement in adults there is a tendency to "conversion to the secondary characters of the opposite sex" Thus adult women with cortical hyper function, generally develop male secondary sexual characters "adrenal virilism" Commonest change noted in the adult male so afflicted is enlargement of breast, sometimes considerable glandular development occurs and may be possible to squeeze out milk from the gland Libido is diminished interest in women decreased, sexual potency and less frequent occurrence of nocturnal erections and seminal emmissions is seen

Gonad Pituitary Relationships

Gonad pituitary relationship has already been established by various workers The chemistry of the Gonadal hormones have been established about 25 years ago Some of them are — (*i*) Oesterone (C_{18} H_{22} O_2) (*ii*) Oestriole, (*iii*) Pregnandiol, (*iv*) Androgens (*v*) Androsterone and (*vi*) Dehydroepiendrosterone (*vii*) Testosterone, and (*viii*) Progesterone The actions of the oestrogens is well known to all of us in normal life In state of oestrus, production of the oestrogen is due to stimulation of the anterior pituitary hormones the F S H During the later stage of pregnancy the physiological activity of the

in animals continues beyond the physiological limit, barring the uterus to seal its os and cervix and disallowing development of the foetus At times it is observed that oestrus will reappear on the next second heat i e after about 42 days of first service or insemination even if the animal is pregnant This condition develops possibly due to the anterior pituitary excess as well as corpus luteal deficiencies

Along with other causes oestrogen level of pregnancy has been observed to be so high at times that prolapse of the vagina repeatedly takes place, though abortion is not effected

The above mentioned syndromes of oestrogenic activities has been attempted to control with the corpus luteal hormone supplementation The condition of suppression of oestrus has been effectively achieved In certain cases of pregnancy, anti-partum prolapse of the vagina which could not be retained permanently by replacement res-

RESPONSE OF PLASMA CORTICOSTEROIDS TO ACTH IN VARIOUS STATES OF ADRENOCORTICAL ACTIVITY*

by PRABHAKER N SHAH, DAMODAR K MAHAJAN and ERNEST J BORGES

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ABSTRACT

I The usefulness of the plasma 17 21-dihydroxy--20-ketosteroid response to intravenously administered ACTH in the assessment of adrenocortical function has been demonstrated

II The adrenocortical response to a standard dose of ACTH in Indian subjects is comparable to that observed in American subjects

III After a standard ACTH test changes in plasma 17-hydroxycorticosteroid levels were helpful in differentiating various etiological categories of Cushing s syndrome and in detecting partial adrenocortical deficiency in apparently normal subjects

It is now well established that hydrocortisone is the predominant adrenocortical hormone in the peripheral blood of primates including Homo Sapiens (Bush) A direct measurement of alterations in circulating concentration of hydrocortisone and some of its metabolites therefore appears to be the most practical approach for a study of the dynamics of adrenocortical activation in man, both in health and disease We are here [illegible]

The first type of adrenal hyperactivity represents the Cushing's syndrome, definable in simplest terms as a sort of symptom-complex due to increased secretion of hydrocortisone The second type of hyperactivity will be described under the adrenogenital syndrome characterised by excessive androgen production without increased hydrocortisone secretion On the other hand, the clinical state of adrenocortical hypoactivity is characterised biochemically by diminution in hydrocortisone and electrolyte regulating factors of the adrenal cortex

[illegible] syndromes

MATERIAL AND METHODS

In all 30 normal subjects of both sexes were selected for the study Of these 27 were females and 3 were males Our criteria of normalcy were

(i) Age range of 16-39 years
(ii) [illegible]
(iii) [illegible]

* This investigation was supported in part by a grant from Mr A V Mody of Unichem Laboratories

(ii) Quantitatively normal menstrual flow and evidence of ovulatory menstrual cycles in females

From each subject 20 ml of blood was drawn in a heparinised syringe These specimens were collected in the morning, between 8-9 a m since there is a rhythmic variation in the levels of plasma corticosteroids — they being at their highest early

for 17 21-dihydroxy-20-ketosteroids

Of the 27 normal females, a standard ACTH test was performed in 6 cases to record adrenocortical responsiveness by infusing intravenously 25 units of ACTH in 500 c c of physiological saline solution over a period of four hours The blood samples for determination of plasma corticosteroids were drawn prior to starting and at the end of this test

In addition, there were 7 patients in whom along with the measurements of urinary

one case

Observation and Comments

Fig 1 represents the range of values of corticosteroids in our subjects It varied

of plasma corticosteroids employed by us is closely similar to that described by these

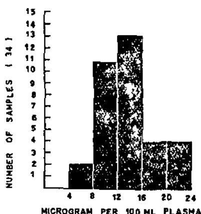


Fig 1

quite different conditions.

Sandberg et al have reported that for unknown reasons there is an elevation in the urinary values of corticosteroids at the time of ovulation. We, therefore, made an attempt to find whether the levels in plasma corticosteroids vary significantly during the two phases of the menstrual cycle. Of the 27 normal subjects having regular cyclic ovulatory bleeding, the determination was made in the preovulatory phase in 12 cases

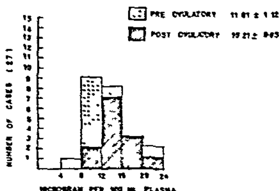


Fig 2

syndrome was a medical rarity 30 years ago, it is now encountered more commonly

syndrome have been now produced in non-endocrine patients by administration of cortisone or hydrocortisone. This is probably the best evidence that Cushing's syndrome is due to excessive production of hydrocortisone.

The various possible causes leading to high production of these hormones are illustrated diagrammatically in Fig 3. Under normal condition, it is considered that ACTH

from the anterior pituitary stimulates the production of adrenal steroids Evidence exists for a 'feed back" system, which means that steroids produced by the ACTH action on the adrenal cortex, act back on the pituitary to control ACTH synthesis Thus there is a reciprocal relation between the anterior pituitary and adrenal cortex and hence neither

POSSIBLE CAUSES OF CUSHING'S SYNDROME

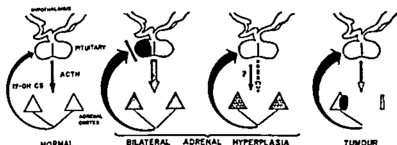


Fig 3

ACTH nor hydrocortisone are produced in excessive amounts in a normal person In Cushing's syndrome, on the other hand, there is always an excessive secretion of hydrocortisone, a [illegible]
about For [illegible]
philism or [illegible]

[illegible]

certain adrenal enzyme systems or is due to excessive secretion of another factor

Table 1

PLASMA 17-OH-CS VALUES IN ADRENOCORTICAL SYNDROMES

	NO OF PATIENTS	17-OH-CS µg/100 ml
CUSHING S SYNDROME	3	31 6 [illegible]
ADDISON S DISEASE	2	
ADRENOGENITA[illegible]	2	

FIGURES [illegible] THE VALUES SOME OTHER





T N K K S.D

Fig 4

from pituitary which potentiates the steroidogenic action of ACTH is still not settled (Grumbach *et al*, 1955, Jailer *et al*, 1956) A third possibility exists where the high production of hydrocortisone may be by the tumour tissue which essentially is independent of ACTH stimulation (Jailer *et al*, 1953) In this particular situation, high circulating level of hydrocortisone on the contrary depresses ACTH production and therefore the other gland gets atrophied

Table I summarises the findings of plasma corticosteroids in the three clinical syndromes It is evident that all the 3 cases of Cushing's syndrome have persistently elevated levels of plasma corticosteroids, while in Addison's disease, plasma corticosteroids are diminished in one case and in the other, it is in the lower range of normal variation In the adrenogenital syndrome, these values fall in the normal range

Fig 4 demonstrates the close resemblance in the clinical appearance of our 3 patients having Cushing's syndrome though the mechanism involved in each may be quite different. One of the most difficult of clinical problems is the differentiation of the Cushing's syndrome arising from hyperplasia of the adrenal cortex from an identical condition caused by a benign or malignant tumour It cannot be overemphasised that a knowledge of the underlying adrenocortical pathology is of practical importance in the therapeutic management of Cushing's syndrome We feel that, of the various tests evolved so far to delineate the various etiological categories in Cushing's syndrome the measurement of hormones from blood and urine is probably the most dependable

Table II presents the alterations in plasma and urinary corticosteroids and urinary 17-ketosteroids in these three patients of Cushing's syndrome It is evident from this table that in each patient the plasma levels of corticosteroids are significantly and persistently elevated The urinary values of 17-ketogenic steroids however do not reflect the true biochemical status in one of these 3 cases The findings of 17-ketosteroids in all the three cases are in the normal range In general, very high 17-ketosteroids in Cushing's syndrome is strongly indicative of adrenal carcinoma but not pathognomonic of an adrenocortical carcinoma since a few such cases having normal levels of 17-ketosteroids are reported

Thus direct measurement of plasma corticosteroids does help in quantitative evaluation of adrenocortical function yet it fails to solve the difficult problem of differentiation

TABLE 2

PLASMA AND URINARY STEROIDAL VALUES IN CUSHING S SYNDROME

PATIENT	SEX	PLASMA 17-OH-CS (µgs/100 ml)	17-KGS * (mg/24 hr)	17-KS £ (mg/24 hr)
T N	F	39 5, (40 0)	20 8	9 9
K K	F	31 6, (30 0)	6 0 (5 8)	3 8 (4 1)
S D	F	30 0, (27 5)	20 0	7 4

* NORMAL EXCRETORY VALUES OF 17-KETOGENIC STEROIDS (17 KGS) OF WOMEN IN OUR LABORATORY ARE 4 10 mg/24 HOUR

£ NORMAL EXCRETORY VALUES OF 17-KETOSTEROIDS OF WOMEN IN OUR LABORATORY ARE 4 - 13 mg/24 HOUR

of various etiological categories of Cushing's syndrome In recent years two approaches, the one anatomical and the other chemical are carried out simultaneously and perhaps would give higher accuracy in differentiating hyperplasia from tumour We, however, believe that the chemical approach is a dynamic one and therefore always preferable to start with The anatomical approach should be employed to locate the pathological lesion or to remove doubts if any still remained after chemical studies have been done The chemical approach appears promising in the light of accumulating knowledge that characteristic responses are recorded following administration of a standard dose of ACTH in different types of adrenal and pituitary diseases

Fig 5 illustrates the individual variations of adrenocortical response as measured by changes in plasma corticosteroids to 25 units of ACTH given intravenously over a period of 4 hours in 6 healthy adult women This response in Indian subjects is comparable to that observed in a comparatively larger group studied by Christy *et al* in U S A It appears that no correlation between the resting level on the one hand and the height to which the steroid concentration rose following infusion, on the other The ACTH test was then performed on our patients having Cushing's syndrome or Addison's disease. [illegible]

levels remain almost unaffected in the remaining two patients It is interesting to mention here that this excessive response seen in K K is after removal of one of the hyperplastic adrenal gland, five years ago

[illegible] it by histo- [illegible] enocortical [illegible] d evidence of presence of pituitary tumour

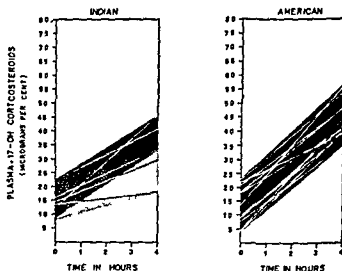


Fig 5

27 5 to 2 5 microgram per cent on the eleventh post-operative day and in the patient T N the level fell from 40 0 to 11 1 microgram per cent during the deep X-ray therapy for pituitary tumour The second part of Fig 6 presents the results of the ACTH test done

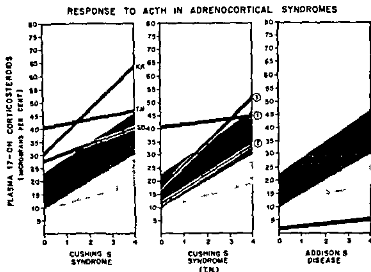


Fig 6

during treatment in the patient T N Curve 1 represents the response before treatment In this state, the adrenocortical cells are working at the maximum capacity because of high or maximal endogenous secretion of ACTH by pituitary tumour Additional exogenous ACTH, therefore, fails to stimulate further production of plasma corticosteroids

also present at this stage Curve 3 depicts the response, 3 weeks after the full tumour dose

and conversely normal adrenal may be present with lower range of normal variation A single low initial plasma steroidal value may therefore be consistent with adequate adrenal function and no rigid interpretation be given unless assessment of the response of adrenal cortex to ACTH is made Early recognition of partial adrenocortical deficiency is of practical importance because under condition of severe stress acute adrenal insuffi-

ACTH The ACTH test, therefore, is the final arbiter in all doubtful cases of Addison's disease It is very pertinent to mention here that saline solution and not 5 per cent glucose in water should be used as a vehicle for ACTH since a full Addisonian crisis has been known to be provoked by the inadvertent use of the latter vehicle

Acknowledgements

The authors are indebted to Dr V R Khanolkar, F N I Director, Indian Cancer Research Centre, for his interest and support of this work Their sincere thanks are due to Dr J C Paymaster, Superintendent, Tata Memorial Hospital for giving them the hospital facilities without which this type of work is not possible

Grateful acknowledgement is made to Dr W J Tindall of Organon Laboratories England, for liberal supplies of ACTH used in this study

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Samuels L T

Ibid 13, 1445

DISCUSSION

Dr Shah from the p cture of Cushings disease which you have shown it seems to me that the case is of the impure type because there are frank indications of hirsutism You may recall that according to Dorfman pure Cushings should not have any symptoms ascribable to excessive androgens

In fact most of the Cushings belong to the impure type

Dr Shah what method did you employ for estimation of plasma 17-OH corticoids?

We used Porter Silber technique which in our hands is giving consistently good results

Dr Shah I am very much interested in your finding about increase in plasma 17-OH corticoid

SECTION C

GONAD — PITUITARY MECHANISMS

THE EFFECT OF OESTRADIOL MONOBENZOATE ON THE OXIDATIVE ENZYMES OF RAT TESTIS

by B CHAUDHURI * A K CHOUDHURY and D P SADHU

Department of Physiology & Nutrition and the Department of Medicine Bengal Veterinary College, Calcutta 37

ABSTRACT

The effect of oestradiol monobenzoate (OMB) has been studied on the oxidative enzymes of rat testis There is an inhibitory effect in the earlier phase which is followed by an increased activity of the succinoxidase system after prolonged injection of OMB The mechanism of this differential action has been discussed

Histopathological changes in the testis such as atrophy and the loss of weight (Hermann and Stein 1916 Spencer *et al* 1932) are observed after administration of oestrogens [illegible]

administration of oestrogens

EXPERIMENTAL

Forty male albino rats from a local strain weighing from 109 to 135 gm were used All the rats were put on the laboratory basal diet for 3 weeks and then divided into two groups

Ten rats served as the control while the other thirty were subjected to daily intramuscular injection of 300 μgm of Oestradiol Monobenzoete (OMB) in oil suspension [illegible] were sacrificed at intervals of twelfth thirteenth [illegible]

were sacrificed simultaneously and determinations of tissue respiration were carried out under identical conditions Tissue suspension was made such that 2 3 ml. of the suspension when added to 0 5 ml of the substrate final concentration of the enzyme preparation in the reaction vessel would be ten per cent This was incubated at 38°C Sodium succinate (0 5 M) and sodium ascorbate (0 114 M) were used as substrate for the determination of the succinic dehydrogenase and the cytocrome oxidase respectively The enzymatic activities were studied by determination of oxygen consumption in a Warburg's apparatus

* Present Address—Junior Scientific Officer Defence Science Organisation Delhi

Results

TABLE 1

Oxygen consumption in (lambda) per mg of wet testis tissue of rat on different days of treatment with 3000 μgm of oestradiol monobenzoate oil suspension

No of animals	Days of Treatment	Without substrate	With Succinate	With Ascorbate
10	0	0 492	0 968	1 212
6	12	0 188	0 332	0 392
6	13	0 188	0 377	0 520
6	15	0 184	0.460	0 588
6	18	0 392	0 704	1.696
6	24	0 433	1 328	2 390

injection of OMB

Discussion

It will be evident from the results of enzyme activity that injection of OMB sets up two different processes in the body, one causing a decrease and the other an increase of the succinoxidase system of the testis Such a phenomenon has not been recorded so far. The amount of depression of enzyme activity at any moment will depend on the

Mc Shan, 1950)

oestrogen would be great enough to destroy its activity completely.

Besides the increased enzyme activity on the twentyfourth day suggests some definite stimulating factor ... nd causes increased secretion ... stimulate the enzyme activit ... eriments are in progress to demonstrate a possible increase in adrenocortical and anterior pituitary

activity as a stimulating factor for succinoxidase activity of rat testis as a secondary effect of *in vivo* oestrogen administration

ACKNOWLEDGEMENTS

[illegible] M Lahiri, Director of Veterinary Services & [illegible] Principal K. C Mukherjee of the Bengal [illegible]

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REGULATION OF GAMETOKINETIC FUNCTION IN INTACT AND HYPOPHYSECTOMIZED MALE TOAD, *BUFO MELANOSTICTUS* SCHNEIDER

by U. K Banik

Indian Institute for Biochemistry and Experimental Medicine, Calcutta

Introduction

Gametokinetic function in male includes both spermatogenesis and spermination The regulation of gametokinetic function in South American toad *Bufo arenarum* Hensel has been studied by Houssay and Lascano-Gonzalez (1929), Burgos (1949), and Burgos and Rufino (1952) Recently, Burgos and Ladman (1957) have studied spermatogenic

mination-test in *B melanostictus* However, precise information is lacking regarding the regulation of spermatogenesis and spermiation in intact and hypophysectomized toad. The present paper relates to the study of gametokinetic function in male toad *B melanostictus*

Experimental Procedure

The common Indian male toad, weighing 4-40 g , were used All animals were maintained in moist wooden box under uniform conditions throughout the period of investigation. Expe

Hypophysectomy

cavity of only adul

... pituitary extract

Spermiation test was performed according to the method of Banik and Chakravarti (1957) All injections were given subcutaneously by single dose

Observations

It will be evident from Fig 1 that testicular weight is reduced after hypophysectomy, which becomes marked only after four weeks Spermatogenesis is also altered progressively

In the seminiferous tubule of adult intact toad all types of germ cells (spermatogonia, ... and spermatozoa) were al... present d...ring the period of

ministration of pituitary extracts (2 mg. wet) twice a week, however, maintained the

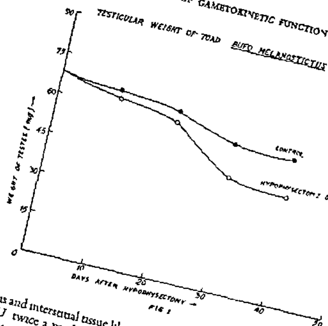


FIG 1

spermatogenesis and interstitial tissue like normal toad up to 30-40 days But the injection of HCG 20 I.U twice a week for four weeks was unable to maintain normal function of spermatogenesis The hormone induced the formation of spermatozoa without any effect on primodial germ cells and as such the lumen of seminiferous tubule was packed with large number of spermatozoa only Other types of germ cells were virtually absent The interstitial elements too remained inconspicuous The same phenomenon was also observed in the lumen of seminiferous tubule in intact animal if 100 I U of hormone was given for five days All germ cells starting from spermatogonia were transformed into spermatozoa through usual successive spermatogenic phases

It will be evident from Table 1 that spermiation in toad induced by HCG remains practically unchanged for 20 days after hypophysectomy and appreciable response is observed even after four weeks

TABLE 1

The influence of HCG on spermiation of Bufo melanostictus *before and after hypophysectomy*

Values are expressed as per cent of positive response

HCG (I U)	Before hypophysectomy	Days after hypophysectomy 10	20	30	40
15	80 0(10)*	77 1(7)	66 6(6)		50 0(4)
20	100 0(11)	100 0(4)	75 0(4)	50 0(4)	50 0(4)
25	100 0(7)	100 0(6)	83 3(6)	63 3(6)	60 0(5)

*Numbers in parentheses indicate the number of animals used in each experiment.

are re
of hc
toad was almost devoid of spermatids and spermatozoa However, 20-25 I U of HCG accelerated the transformation of spermatogonia through chronological phases to spermatozoa and eventually the spermiation It is also evident from Table 2 that small sustained doses of the hormone (2 5 I U /day for 4 days) do not evoke spermiations although the tempo of spermatogenesis starting from spermatogonia was found to be accelerated

TABLE 2

Influence of HCG on the spermiation of immature intact toad Bufo melanostictus

Toads weighing 4 10 g were used HCG was injected at random and sex was determined by sacrificing the animals after completion of the experiments

HCG (I U)	Percentage of positive response
2 5	0 0(7)
5 0	10 0(10)
10 0	40 0(10)
15 0	60 0(10)
20 0	100 0(10)
25 0	100 0(7)
2 5/day for 4 days	0 0(10)

*Numbers in parentheses indicate the number of animals used in each group

The effect of HCG in gametokinetic function in toad seems interesting and different from mammalian male Simpson *et al* (1951) believed that HCG and interstitial cells

comparable to that obtained by administration of pituitary gonadotrophin However

This study shows that while spermiation could be induced by HCG the complete spermatogenesis in *B melanostictus* is not regulated by HCG alone

ACKNOWLEDGMENT

The author is grateful to Dr J C Ray, F N I for his keen interest and constant encouragement in the work Thanks are due to Mr B Basak and Mr N C Mitra for technical assistance and to Miss Anita Barua for her help in many ways

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STUDIES ON THE EFFECT OF ADMINISTRATION OF GONADOTROPHINS IN AUGMENTING FERTILITY IN FARM ANIMALS

by P Bhattacharya, F N I and S N Luktuke

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Abstract

Cows and heifers with inactive ovaries were in ected with varying doses of PMS Oestrus wa[s] induced in most of the treated cows within 2—4 days after treatment Pregnancies were obtained in 22 out of 28 animals as a result of administratio i f PMS

PMS was administered to 102 buffalo females in anoestrum various stages of oestrous cycle and early pregnancy Multiovulations occurred when the hormo ie was injected during the follicular phase of the oestrous cycle and during the mid-oestrous cycle in the presence of corpus luteum which was subsequently enucleated at various intervals after treatment Single ovulation occurred in a large number of the anoestrous animals The ovaries of the pregnant a imals treated with PMS were considerably enlarged The ovarian weight varied from 17—82 gms

Rams were treated with PMS with a view to determining the effect of administration of t[he] hormone during summer when the atmospheric temperature is high and humid and when the quali[ty] of semen is comparatively poor Semen obtained from the experimental animals was of a superio[r] quality than that obtained from the treated ones Buffalo bulls similarly treated with PMS did no[t] give satisfactory response

The discovery of a potent gonadotrophic hormone by Cole and Hart (1930) in the blood serum of pregnant mares led to a considerable amount of research on the possibility of augmenting fertility in livestock by hormone administration Cole and Miller (1933) were first to report the effect of administration of gonadotrophic hormones on anoestrous sheep They could induce ovulation in the quiescent ovary of the anoestrous ewe by a single or repeated injections of equine gonadotrophin but although ovulation was obtained it was generally unaccompanied by oestrus With this work was initiate[d] a series of investigations in various species These studies were reviewed by Luktuk[e] (1954)

Hammond and Bhattacharya (1944) conducted an elaborate investigation on the control of ovulation in the cow by hormonal methods They demonstrated that superovulation could be obtained by injections of horse pituitary extracts or PMS if the corpus luteum was regressing or was manually removed Folley and Malpress (1944) injected cows with PMS in the luteal phase of the cycle Though the development of follicles was stimulated, no ovulation occurred

Experiments and clinical observations on the use of gonadotrophins [illegible] of functional infertility in cattle have also been reported by various w[illegible] *et al* 1940, Bhattacharya *et al* 1941, Asd[illegible] 42 Cameron, 19[illegible] Luktuke and Bhattacharya, 1948 Abma[illegible]

Effect of administration of gonad[illegible] ovaries and in fecundity in cattle was studied by C[illegible] Folley and M[illegible] Hammond and Bhattac[illegible] (1944) T[illegible] al u[illegible] lation in cattle has [illegible] by [illegible] (1949) Umbaugh (19[illegible] (19[illegible] acharya suitable conditions [illegible] ons can [illegible] ions ha[illegible] as fifty follicles ovu[illegible] e in [illegible] estic ani[illegible] 1949)

multiovulation has become a problem of great importance in researches pertaining to the transplantation of fertilized ova in cattle Casida *et al* (1943) stimulated calf ovaries by anterior pituitary gonadotrophins Marden (1953) and Black *et al* (1953) attempted to exploit the juvenile calf ovary for the production of viable ova

In India the intercalving period on an average is very long and a large proportion of cows take a long time to come to heat after parturition An attempt was therefore made to reduce the intercalving period by inducing oestrus and ovulation with PMS These investigations are reported in Experiment No I As in cows a condition known as anoestrus characterised by marked gonadal subactivity possibly due to the lack of stimulation of the hypophysis is commonly encountered in buffaloes as well Delay in getting these animals in calf and the resultant loss in milk yield necessarily constitutes a serious problem During a countrywide survey on bovine infertility conducted from the Division of Animal Genetics Indian Veterinary Research Institute it was found that 66% of buffalo heifers over 3½ years age and 53 per cent of buffalo cows which had calved more than 4 months back had subactive ovaries (Bhattacharya 1954) The investigations reported in Experiment No II in this paper were aimed at inducing oestrus and ovulation in anoestrous buffalo with equine gonadotrophin and for studying the effect of administration of the hormone during various phases of the oestrous cycle and early pregnancy in this species The study was thus also concerned with the potential fertility of eggs that have been ovulated by artificial means

The quality of semen produced by bulls buffalo-bulls rams and goats deteriorates during the part of the year when air temperature is high coupled with high humidity (Mukherji and Bhattacharya 1952 Shukla and Bhattacharya 195- *a b* Kushwaha *et al* 1955) Studies concerning the attempts made to improve the semen quality in rams (Luktuke 1954) and in buffaloes (Goswami 1957) by activating the testes with the administration of PMS in unfavourable season have also been included in this paper (Experiments III & IV)

MATERIAL AND METHODS

Experiment No I

Experimental animals for this study consisted of 34 cows and 3 heifers The cows were anoestrous for over four months after parturition The heifers though long mature were anoestrous The animals were divided in two groups In the first group 9 cows were injected with 1000-2000 I U PMS These animals were slaughtered 4 10 days after injection In the second group comprising of 25 cows and 3 heifers the dosage of PMS administered varied from 1000 to 3000 I U Eight cows from this set were slaughtered after a period of 2 months or over All the experimental animals were either artificially inseminated or were given a natural service at the induced or subsequent heats

Experiment No II

Experimental animals for this study consisted of 102 buffaloes including 10 buffalo-heifers and three buffalo-heifer calves Twelve buffalo cows were purchased from the local cattle markets Six buffalo cows were treated at the Military Dairy Farm Bareilly and fifteen animals were offered by private cattle owners for treatment The remaining animals used in this investigation were obtained through an arrangement made with the local abattoir agents whereby buffaloes destined for slaughter were temporarily kept at the Institute for experimental purposes These animals were almost of uniform age-group and were in good condition of health

Out of the 12 purchased animals, eleven were slaughtered in the Institute's post mortem rooms and one was later auctioned on her becoming pregnant The animals obtained on contract were maintained at the Institute for observations and treatment and were slaughtered at the local abattoir Six animals at the Military Dairy Farm, Bareilly and 15 animals offered by private cattle owners, were all anoestrous These animals were [illegible]

naturally at the induced heat and were left to calve The private cattle owners were requested to present their animals for insemination as soon as oestrum was noticed after treatment

All injections of PMS were given subcutaneously in the neck region, with a light massage for a few seconds after the injection The dosage varied from 750 I U to 6000 I U

After injections, the animals were frequently examined for the onset of oestrum and the examinations were carried out at still lesser intervals till the animals went off heat All animals except those at the Military Dairy Farm, Bareilly were inseminated during the declining phase of the oestrus The inseminations were repeated after 18 hours if the animals continued to remain in oestrus for more than one day Semen from two buffalo bulls was used throughout for inseminating animals in this investigation The quality of semen was good and contained at least 80 per cent motile spermatozoa

Examination of material

Animals were slaughtered at appropriate times at the local municipal slaughter house (except the eleven purchased animals mentioned above, which were destroyed in the postmortem rooms of the Institute) and after slaughter, the reproductive organs were removed as quickly as possible and were brought to the laboratory wrapped in wet towels for investigation

In the laboratory, the organs were carefully dissected and the following particulars were recorded

1 Weights of ovaries and uterus
2 Ovulation points
3 Number of unovulated follicles
4 Number of old corpora lutea and luteal scars
5 Condition of uterus, cervix and vagina

The diameters of follicles and corpora lutea were measured with vernier callipers

The follicles were classified as

1 Very large (VL) — Over 15 mm in diameter
2 Large (L) — Between 10-15 mm ,, ,,
3 Moderate (M)— , 5-10 , ,, ,,
4 Small (S) — ,, 2-5 , ,, ,,
5 Very Small (VS) — less than 2 mm in diameter

Estimate of the approximate age of all induced corporalutea were made from their size and colour

Recovery of ova

In case of animals in which ovulation had taken place, the fallopian tube on the side

of ovulation was carefully dissected to straighten it out. Warm physiological saline solution was rinsed through the fallopian tube carefully with the help of a glass syringe into medium sized flat bottomed watch glasses. In every watch glass about 20 drops were drained out. As a routine washings were obtained into 6–8 watch glasses. These washings were soon examined under microscope for the recovery of ova and the evidence of cleavage. The ovum, if present in the oviduct, usually came out with the first few drops of fluid. The diameter of the ovum was measured with the help of a micro-meter.

Classification of groups

The animals were grouped as shown below

Sr No	Group	Buffalo heifers	Buffalo Cows	Total
1	Injection of PMS in anoestrous animals	9	28	37
2	Injection of PMS during mid oestrous cycle in the presence of a well developed corpus luteum		6	6
3	Injection of PMS during mid-oestrous cycle and corpus luteum removed at various intervals thereafter		10	10
4	Injection of PMS at various intervals after the enucleation of mid-oestrous cycle corpus luteum		4	4
5	Injection of PMS simultaneously with the enucleation of corpus luteum		6	6
6	Injection of PMS during follicular phase of oestrous cycle		11	11
7	Injection of PMS during early pregnancy		6	6
8	Anoestrous animals treated with PMS and left to calve	4	18	22
	Total	13	89	102

Experiment No. III

Ten rams were divided into two equal groups on the basis of their semen production. One group received during summer three subcutaneous injection of 400 I.U. of PMS at intervals of 2 weeks. Two ejaculates of semen were obtained in quick succession at weekly intervals from each animal. The semen samples were examined for different attributes.

Experiment No. IV

Eighteen buffalo bulls were divided into three equal groups. Animals of one group were administered PMS in doses of 1000 I.U. per week, those in the second group were given per os 100 mg. of sodium thyroxine per day. The treatment period lasted for 14 weeks. The semen from all the bulls was examined at regular weekly intervals for various attributes.

RESULTS

Data collected on the ovarian response to administration of PMS of nine cows slaughtered in the first group of Experiment I are presented in Table No. 1. Single ovulation occurred in six cows and twin ovulations in one with PMS treatment.

Pregnancies were obtained in 2 out of 8 animals of the second group in Experiment I as a result of treatment with PMS. Data are presented in Table No. 2.

The results of experiment No. II are summarised in Table Nos. 3, 4 and 5.

Administration of PMS in subactive condition of ovaries caused single ovulation in a majority of animals In the present investigation twin ovulation was observed in only two anoestrous buffaloes It has been observed that when the anoestrous condition is very deep and the ovaries are atrophied, even large doses of PMS have failed to develop follicles

There was no incidence of multiovulation in this group of animals The first follicle which attained maturity probably ovulated spontaneously and the formation of new corpus luteum inhibited ovulation of other follicles According to Hammond (19 7) ovulation occurs in bovines in follicles which are 10-15 mm in diameter In an investigation on the development of follicle and corpus luteum in the buffalo Luktuke and

over 10 days and no ovulation occurred Examination of ovaries by rectal palpation

Folley and Malpress (1944) as Hormone Hypertrophy

Pituitary activity in calves and in very young heifers is at a much lower level compared to the adult females and hence the mode of response is expected to be somewhat different Casida *et al* (1943) experimented with 9 calves of unknown age and obtained 129 follicles but only 9 corpora lutea Marden (1953) has shown that ovarian stimulation can be induced in the juvenile bovine through the administration of gonadotrophic extracts Both these workers have found that relatively few ova are recoverable from calves in relation to the number of corpora lutea present The percentage of fertilized ova recovered is also low

In the present study only 3 heifer calves and 6 heifers were treated with PMS Ovulation could be induced only in one heifer calf As the heat symptoms were not evinced the animal was not artificially inseminated The ovum recovered from this animal was therefore unfertilized Out of the 6 heifers 3 ovulated and in two of them the ovum was fertilized

Casida *et al* (1943) made attempts to induce superfecundity in the cows by treating them in the luteal and follicular phases of the oestrous cycle No pregnancies were obtained in the cows when treated during the luteal phase but a normal percentage of pregnancies occurred in the animals in which treatment was given in the follicular

(15th day) of the oestrous cycle a single subcutaneous injection of 2000 I U PMS was given and followed by an intravenous injection of sheep pituitary extract five days later All the sows were mated or artificially inseminated at the time of intravenous injection and slaughtered 48-56 hours later

Fertilized ova were not found in any of the 'luteal sows' although an average of 10 6 ova was recovered per sow In the follicular group, 19 6 ova per sow were recovered and of these 5 3 were fertilized In the investigation conducted by Tanabe *et al* (1949) with 22 sows and 45 gilts, there was also a very low incidence of fertilization of the ova produced, during the luteal phase of the oestrous cycle

Hammond and Bhattacharya (1944) treated 17 cows with varying doses of PMS, during the follicular phase of the oestrous cycle They obtained multiple ovulations in two ways, either by injection of PMS towards the end of normal cycle or by its administration earlier in the cycle and subsequent expression of the corpus luteum Ovulation normally occurred only after removal or regression of corpus luteum present at the time of injection Multiovulations were produced in 7 of these cows

Gonadotrophins have been used to produce superovulation in rats and mice. There was relatively a high rate of mortality in the new borns but larger than normal size of litter was produced (Engle 1927, Cole 1927 Evans and Simpson 1940) Thus problem of superovulation was also investigated in both cattle and sheep (Casida *et al*, 1943, 1944) Response obtained varied with the gonadotrophins used and the stage of oestrous cycle at which the treatment was given (Murphree *et al* 1944)

Hammond and Bhattacharya (1944) and Hammond (1949) induced twin and multiple ovulation in cattle by the use of PMS given as a single subcutaneous injection Various methods to increase the number of viable ova shed by the cow have been tried (Dowling, 1949, Hammond, 1950, Rowson 1951 Brock and Rowson 1952) Dowling (1949) achieved considerable success by using three daily injections of 100 mg of horse pituitary extract during the follicular phase of the cycle Injection of 3000 I. U of PMS subcutaneously at any time between 4-10th day of oestrous cycle was found by him to result in marked follicular stimulation and a high ovulation rate (81 per cent) after L H was given intravenously However, none of the ova were fertilized Casida *et al* (1943) had previously shown that ova shed during the luteal phase of oestrous cycle were not capable of fertilization

The technique of induction of multiovulations is of great practical importance in studies pertaining to the transplantation of fertilized ova (Chang, 1949, Umbaugh, 1949, Hammond, 1950, Lamming and Rowson 1952) In the present investigation, multiovulations were induced in buffaloes by injecting PMS during mid-oestrous cycle and the corpus luteum was removed at various intervals after injections. Twentyseven ovulations were obtained from nine animals Out of the 17 ova that were recovered only 3 were found to be fertilized (17 65 per cent) Multiovulations have also been induced in 9 other buffaloes by treating them with PMS during the follicular phase of the oestrous cycle 24 ovulations were obtained from 9 animals and out of the 16 ova which were recovered, 14 were at various stages of development (87 50 per cent) Casida (1943) and Rowson (1951) had found while inducing superovulation in mature animals during luteal phase, that the resulting fertility is exceedingly low Willett *et al* (1952) have tried to deposit the semen in the uterus of cow during the luteal phase of the cycle and found that fertility continued to remain low

Robinson (1950, 1951) has obtained promising experimental results in raising the level of fertility in ewes in the breeding season, so that a high percentage of twins are born This has been obtained by injecting the ewes with 450 I U of PMS on the 12th day of oestrous cycle (follicular phase)

A number of follicles mature during oestrous. The follicles which are unovulated

TABLE 1

Ovarian response of cows to administration of PMS

Sl No	Animal No	Dose i u	Interval in days between		Follicles						Approx. time of ovulation	Weight of			Remarks
			Inj and heat	Inj and slaughter	VL	L	M	S	VS	H	Days after injection	Uterus	R O (mg)	L.O	
1	8	1000	—	8	—	1	3	—	—	—	3	121.4	3 09	3 0	Ovulation L.O
2	11	1500	—	8	—	14	1	—	—	—	4	114 5	23 7	15 7	L O
3	22	1000	—	8	—	8	1	20	2	—	—	116 5	12 5	12 5	No ovulation Old C L. present in L O
4	24	1000	6	9	—	—	2	2	—	2	7	93.0	3 4	1 9	Ovulation L O Ovum recovered
5	62	1500	4	8	6	10	5	—	—	—	6	131 6	14 8	13 5	Ovulation L.O
6	63	1500	3	7	1	9	3	2	—	—	—	98 0	8 3	3 8	No ovulation Very small old C L present in L O
7	65	1200	—	10	—	2	5	21	9	—	5	113 0	4 0	5 5	Ovulation R O
8	66	1200	2	8	13	14	7	5	8	—	8	116 6	22 1	31 1	Two ova recovered. One ovulation in each ovary
9	72	2000	3	4	1	6	5	7	13	—	4	103 5	5 5	10 4	Ovulation L O

R.O — Right Ovary
L.O — Left Ovary
C.I — Corpus luteum
H. — Haemorrhagic
VL. — Very Large
L. — Large
M — Moderate
S — Small.
V S — Very Small

TABLE 2

Effect of PMS n restoring fe t l y i cows & he f rs

Sl No	Animal No	Breed	Dose i u	Mode of Administration	Interval in days between: Inj and 1st heat	Interval in days between: 1st and 2nd heats	Result	Remarks
	COWS							
1	2 —	non descript	1200	Subcutaneous	46	24	Pregnant	Slaughtered N foetus recovered
2	4		1200		28	—		
3	5		1000		2	—		
4	7		1600		2	—		
5	8		2000		2	—		
6	10		2000		2	—		
7	16		1200		4	—		
8	25		1000		2	—		
9	1		1200		45	—		Calved at term
10	15A		1200		2	—		
11	21A		1600		4	97		
12	15		1500		3	—		
13	120	Hariana	1500		4	18		
14	185		1500		4	22	*Repeated	D ed
15	344		1500		11	169	Pregnant	Calved at term
16	12	on-descript	1000	Intra muscular	9	—		Aborted (two months)
17	16		1200		2	22	*	Calved at term
18	19		1000		2	68		
19	19D	Sahiwal	1500	Subcutaneous	5	56	Repeating	
20	96D	Hariana	1000		3	22	Pregnant	Carrying
21	182D		1200		2	39	Repeating	
22	39	non-descript	3000		4	18	Pregnant	Carrying
23	44		3000		3	18	Repeating	
24	48	"	3000		3	25	Repeating	
25	10		3000		3	9	Pregnant	Carrying
	HEIFERS							
26	18	Gir	1500		8	25		
27	P1	non-desc pt	1600		4	—	*Not Pregnant	
28	P112		1000		6	19	Pregnant	Calved at term

*Conceived after repeating a number of times
N — Normal

TABLE 3

Sl No	Animal No	Age Yrs	Body weight lb	Dose of PMS I U	Interval (in days) between injection and Heat	Slaughter	Weights (in g) Uterus	R.O	L O	Ovulations R O	L O	Ova recovered	State of Development of ovum
					Injection of PMS in heifer calves								
1	13	1½	308	1000	4	5	120	0 5	0 7	—	—	—	—
2	14	1½	394	1000	—	5	100	1 0	0 7	—	—	—	—
3	52	2	392	3000	—	8	89	8 4	14 8	1	—	1	Unfertilized
					Injection of PMS in heifers								
1	16A	6	390	1200	—	12	196	1 7	1 8	—	—	—	
2	23	6	508	3000	3	7	203	3 2	12 6	1	—	1	4 cell stage
3	27	7	502	3000	3	8	179	13 1	3 3	—	1	1	16 cell stage
4	28	7	573	3500	—	7	155	1 3	0 8	—	—	—	
5	55	4	532	3000	—	10	195	7 2	9 2	1	—	1	Unfertilized
6	17	8	588	3000	—	7	240	14 0	9 0	—	—	—	—

TABLE 4

I ject on of PMS i i a ce trous b ffalo cou s

Sl No	Animal No	Body weight lb	Dose of PMS I U	Interval (in days) between inject on and Heat	Slaughter	Weights (in g) Uterus	R O	L O	Ovulations R O	L O	No of ova recovered	State of Development of ovum
1	9A	760	1500	4	7	47	2 5	6 4	1	—	1	2 cell stage
2	11A	830	1500	4	6	135	1 7	2 0	—	1	—	
3	12A	740	1500	4	7	2(1	8 8	4 4		—	1	Unfertilized
4	14A	650	1 00	7	12	34	1 0	1 9	—	1	1	Unfert lized
5	13A	500	1200	5	7	135	1 9	1 5	1		1	Unfertilized
(	18A	650	1200	6	9	268	6 6	6(	1	—	—	—
7	19A	450	1200	8	26	199	2 1	2 4	—	—	—	—
8	20A	472	1200	(	)	22	3 1	4 7	—		1	2 cell stage
)	21A	500	1500	8	26	145	1 5	1 4	—	—	—	—
10	23A	550	2000	4	11	282	6 3	2 9	—		—	—
11	25A	515	1500	3	20	182	1 8	1 1	1		—	—
12	3	812	1500	3	7	455	11 3	16 3	1	—	1	U fertil zed
13	6	700	1500	3	6	460	4 2	13 4	—	1	—	—
14	8	640	1500	4	7	299	18 2	7 5	—	—	—	—
15	10	560	1500	2	6	146	2 2	6	—	1	1	8 cell stage
16	16	768	1500	6	10	329	5 0	11 9	—	—	—	—
17	21	702	1500	2	7	118	3 2	5 0	—	1	1	4 cell stage
18	22	585	3000	2	(	196	1 9	0 9	1	—	1	*Unf rtilized*
19	25	619	4500	4	9	357	14 2	4 5	—	—	—	—
0	36	512	3000	3	8	257	1(1	6 3	1	—	1	Unfert lized
21	43	784	4500	3	8	405	4 8	5 2	—	1	1	2 cell stage
22	44	(88	4500	3	7	273	16 2	21 0	1	—	1	Unfert l zed
23	45	616	4500	3	6	340	14 1	13 7	—	—	—	—
24	50	560	4500	3	7	315	11 8	13 9	—	1	1	16 cell stage
25	77	868	3000	5	11	474	7 7	9 0	—	—	—	—
6	80	674	4500	7	12	391	1 0	13 0	—	—	—	—
27	81	804	3000	3	7	356	5 7	7 0	1	1	2	4 cell stage
28	17B	772	4500	3	7	390	12 9	9 8	1	—	1	3 4 cell stage

TABLE 5

Treatment of buffalo-cows with Pregnant Mare Serum Gonadotrophin (PMS) and the recovery of ova

Sl No	Group	No of animals treated	No of animals ovulated	No of ovulations			No of ova recovered			Percentage recovery of ova	No of fertilized ova			Percentage of fertilized ova recovered
				R	L	T	R	L	T		R	L	T	
1	IA	28	18	12	9	21	9	7	16	76 19	3	6	9	56 25
2	IB	3	1	1	—	1	1	—	1	100 00	—	—	—	00 00
3	IC	6	3	2	1	3	2	1	3	100 00	1	1	2	66 67
4	II	6	—	—	—	—	—	—	—	—	—	—	—	—
5	III	10	9	10	17	27	8	9	17	63 00	2	1	3	17 65
6	IV	4	3	4	1	5	4	1	5	100 00	4	1	5	100 00
7	V	6	4	2	2	4	2	2	4	100 00	2	2	4	100 00
8	VI	11	9	13	11	24	10	6	16	66 67	9	5	14	87 50
9	VII	6	—	—	—	—	—	—	—	—	—	—	—	—
Total		80	47	44	41	85	36	26	62	72 94	21	16	37	59 68

R Right ovary
L Left ovary
T. Total

IA Injection of PMS in anoestrous buffalo cows

IB Injection of PMS in anoestrous heifer calves

IC Injection of PMS in anoestrous heifers

II Injection of PMS during midoestrous cycle in the presence of a well developed corpus luteum.

III Injection of PMS during mid-oestrous cycle and corpus luteum removed at various intervals thereafter

IV Injection of PMS at various intervals, after the enucleation of mid oestrous cycle corpus luteum

V Injection of PMS, simultaneously with the enucleation of corpus luteum

VI Injection of PMS during follicular phase of oestrous cycle

VII Injection of PMS during early pregnancy

TABLE 6

Average of semen qualities of treated and untreated rams during the experimental period—1st Ejaculate

	Control group	Experimental group	Difference between two means	t value	Significance level
(i) Volume of semen (ml)	0 60	0 69	0 09	1 91	Not significant.
(ii) Sperm concentration (millions/ml)	2841	3814	973	3 10	1%
(iii) Total No of spermatozoa (millions/ejaculate)	1845	2792	947	5 04	2%
(iv) Percentage of abnormal spermatozoa	11 09	6 26	4 83	3 00	2%
(v) Initial fructose (mg/100 ml of semen)	583	511	72	1 89	Not significant
(vi) Fructolysis (%)	54	72	18	2.46	5%

TABLE 7

Averages of semen qualities of treated and untreated rams during the experimental period—2nd Ejaculate

	Control group	Experimental group	Difference between two means	t value	Significance level
(i) Volume of semen (ml)	0 47	0 59	0 12	2 31	5%
(ii) Sperm concentration (millions/ml)	2326	3531	1205	4.46	1%
(iii) Total No of spermatozoa (millions/ejaculate)	1231	2296	1065	4 43	1%
(iv) Percentage of abnormal spermatozoa	13 07	6 89	6 18	3 99	1%
(v) Initial fructose (mg/100 ml of semen)	647	533	114	2 72	2%
(vi) Fructolysis (%)	39 46	65 15	25 69	3 99	1%

TABLE 6

Range of Variation and the Mean of Reaction-Time and Semen Characteristics of Buffalo Bulls during the Pretreatment, Treatment and the Post-treatment Periods

Reaction time, and Semen Characteristics	Group	Pretreatment period		Treatment period		Post-treatment period	
		Range	Mean	Range	Mean	Range	Mean
Reaction Time in Seconds	C	18 0-59 8	38 00(34)	10 5-34 3	18 6(83)	14 8-43 5	23 0(76)
	TH	14 6-22 1	19 85(35)	11 0-28 6	17 6(81)	9 3-54 0	22 9(78)
	PMS	16 8-45 3	28 83(36)	11 3-44 3	24 7(83)	9 1-42 6	22 2(76)
Volume	C	1 24-2 09	1 81(35)	1 62-2 54	1 99(83)	1 20-2 16	1 76(76)
	TH	1 21-2 32	1 66(35)	1 08-2 12	1 55(81)	0 87-2 16	1 38(78)
	PMS	1 17-2 15	1 69(36)	1 15-2 42	1 81(83)	1 31-2 50	1 82(76)
Initial Motility of Spermatozoa	C	1 5-2 7	2 07(35)	2 0-32	2 70(81)	2 0-3 4	2 75(72)
	TH	2 0-2 5	2 20(35)	2 2-3 4	2 92(80)	2 5-3 7	3 28(75)
	PMS	1 4-2 3	1 95(06)	1 9-3 4	2 73(13)	2 2-3 6	3 00(74)
Sperm concentration in millions/ml	C	536 6-796 0	721 0(35)	418 0-870 8	605 0(81)	231.6-1333 0	726 5(72)
	TH	575 8-879 1	713 6(35)	355 8-835 8	587 8(80)	481 6-1394 1	571 0(75)
	PMS	505 0-909 1	677 1(36)	340 0-808 0	538 1(83)	283 3-972 5	630 3(74)
Total sperm in millions/ ejaculate	C	710 9-1861 5	1321 2(35)	607 2-1638 5	1154 7(81)	346 3-2623 6	1418 6(72)
	TH	553 3-2153 0	1275 0(35)	496 8-1558 9	1029 2(80)	856 7-1950 7	1252 5(75)
	PMS	775 7-1887 8	1257 8(36)	509 5-1480 7	1020 9(83)	432 8-2077 8	1201 0(74)
Initial fructose content in mgm/100 ml of semen	C	472 2-592 6	543 4(25)	664 3-950 2	821 9(82)	793 6-1054 4	902 6(71)
	TH	551 3-592 8	578 3(25)	767 9-943 9	839 8(80)	755 7-1102 9	872 5(76)
	PMS	394 3-591 9	556 9(30)	615 3-896 5	777 5(83)	730 6-872 6	820 11(74)
Fructolysis Index	C	0 99-3 52	1 96(17)	1 12-3 30	1 98(64)	0 55-4 56	1 98(53)
	TH	0 74-2 76	1 93(24)	1 52-3 49	2 31(76)	1 04-3 20	2 03(67)
	PMS	0 70-3 49	1 81(27)	1 06-2 35	2 11(61)	1 21-3 49	2 07(62)
%of abnormal spermatozoa	C	was not estimated		9 89-25 86	15 82(81)	12 01-20 51	17 09(67)
	TH			6 42-22 41	11 83(80)	7 42-10 07	8 60(71)
	PMS			11 05-29 25	18 89(83)	11 05-28 29	17 98(70)

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CLINICAL REPORTS ON TESTICULAR HYPOPLASIA IN GOVERNMENT BREEDING BULLS OF WEST BENGAL

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ABSTRACT

Observations on some clinical cases of hypoplastic testes in Hariana bulls have been reported Two of these animals showed bilateral hypoplasia while the other two showed unilateral hypoplastic testes. It was observed that the secondary sex characters and organs were apparently normal in unilateral hypoplasia but in cases of bilateral hypoplasia there was definite underdevelopment of secondary sex characters and organs and feminisation of the bulls

The problem of ster l n and nfert l ...

ficial Insemination work, has not so far been appreciated Extensive research has been made in Europe and America on the subject of sterility and infertility due to Hypoplasia in bulls by eminent workers like Williams, (1947) Lagerl of (1938) and many others

Here in the Eastern Hemisphere we consider the question of infertility and sterility ...

power or become responsible for the production of defective offsprings

... r as a cow" So far as infertility ... be one of the commonest causes ... It may be unilateral or bilateral ... ıys

The present work describes the results of some clinical observations on some Govt bulls distributed for breeding purposes

Investigation —

... to follow up the case for detailed Morphological, Histo-pathological and other examinations Occasional cases of hypoplastic testes encountered in the animals of other breeds have not been considered here

The study consisted of clinical examinations of the primary and secondary sex organs and histopathological examinations of some of these organs after death

Out of such 20 Hariana bulls examined, 4 cases of Hypoplastic testes were detected Of these 4 cases, 2 were of bilateral in nature and the other two of unilateral type—the right testes being the affected one

Observations —

I Bilateral hypoplastic teste s —

(a) Clinical symptoms—The testes were much smaller than the usual size They were unduly hard with a roundish contour It was extremely difficult to locate the tail of epididymis on palpation The spermatic cords were very short and distorted

(b) Morphology—The testes were highly underdeveloped in consideration of breed, size and weight of the animals (about 700 lbs) and age (5 years) and weighed for 60-80 Gms

(c) Histo-Pathology—Majority of the seminiferous tubules were vaculated Different formative stages of the spermatozoa could not be seen Most of the tubules were devoid of spermatozenic epithelium and paratubular fibrosis though present was not very prominent

(d) *Secondary Sex organs and characters—Animals presented a feminine appearance* with slender neck and small head and showed a feminine temperament The secondary sex glands e g seminal vesicle prostrate, etc were comparatively smaller, though the penis showed almost normal development,

II Unilateral Hypoplastic testes —

(a) [illegible]

Hyperplasia of the epididymis of the other side circumscribed by an unusually clear limiting line

(b) Morphology—The normal testes with apparent hypertrophy of the epididymis was suggestive of its tendency to carry on some compensatory function But the size of the normal testes did not show any metaplastic changes, spermatic chord was shorter on the affected side

(c) Histopathology—The hypoplastic testes was found to have reduced cell layers and *in hypofunctioning state*

(d) Secondary sex organs and characters—Development of the secondary sex organs was not affected Serving ability, secondary sex characters and fertility appeared to remain mostly unimpaired

Discussions

[illegible]

According to Erikson (1943) testicular hypoplasia is caused by a recessive autosomal gene with incomplete penetrance His earlier finding has been confirmed by wide genetic investigations (1950)

The scorbutic guinea pigs thus showed lowered glucose tolerance and they deposited less glycogen in the liver. These observations suggested diminished insulin secretion in the scorbutic animals. Insulin content of the pancreas of scorbutic guinea pigs as well as the paired fed normal guinea pigs were extracted and assayed by the rabbit method of insulin assay. The results are given in Table III.

TABLE III

Insulin content of pancreas of guinea pigs

Animals	Weight of pooled pancreas (gm)	Insulin content per gm pancreas (International Units)
Normal (10)	11 91	0 45
Scorbutic (10)	10 38	0 11

Figures in parenthesis indicate the number of animals.

The insulin content of the pancreas was markedly diminished when the animals developed scurvy.

[illegible]

from the tail end of each pancreas, stained with iron hematoxylin, the number of islets was counted and the size of the individual islets was measured (Banerjee, 1944) The results are given in Table IV.

TABLE IV

Number and size of islets of Langerhans of pancreas of guinea pigs

Animals	Number (mean) of the islets in each setion	Total (mean) size of the islets in each section (sq mm)
Normal (10)	7 6	273.52
Scorbutic (10)	11 2	1168 25
t	3 09	2 1

Figures in parenthesis indicate the number of animals

The sections which were stained with azan stain were examined for the different types of cells present in the islets of Langerhans. In the scorbutic guinea pigs, alpha cells were found to be increased in number in proportion to the beta cells, and the beta cells were found to be mostly degranulated. [illegible] change in the sections studied The increase in the size and als[illegible] attempt to react against the fall in insuli[illegible] any *degenerative changes seems to be* [illegible] place when the scorbutic guinea pigs are given supplements of vitamin C.

[illegible] ...lished it was [illegible]

Guinea pigs were separated into several groups, each group consisting of one normal, one scorbutic and one insulin treated scorbutic guinea pig. The guinea pig in each group was fed equal amounts of the scorbutic diet. Regular insulin was injected subcutaneously into the animal intended for insulin treatment with a dose increasing from 0.1 to 0.3 unit per 100 gm. of body weight per day from the beginning of the second week. On the day of the experiment insulin injection was stopped. An oral glucose tolerance was made in the 4th week of the regime with the scorbutic diet on eleven groups of animals after they were fasted for 15 to 18 hours. Animals were killed after the glucose tolerance test and liver and muscle were removed for the glycogen estimation (Banerjee, Biswas and Singh, 1958). The results are given in Tables V and VI.

TABLE V

Glucose tolerance test in guinea pigs after different treatments

Animals	Fasting blood sugar (mg %)	Blood sugar (mg %) after glucose feeding			
		45 min	90 min	150 min	180 min.
Normal (11)	111±4.6	154±11.6	181±13.1	144±10.4	123±11.0
Scorbutic (11)	124±5.3	197±17.0	244±18.4	248±18.8	227±17.6
Insulin treated scorbutic (11)	128±4.4	226±16.5	242±18.9	198±7.9	161±9.8

Figures in parenthesis indicate the number of animals.

TABLE VI

Glycogen content of liver and muscle of guinea pigs (gm./100 gm. fresh tissue)

9 normal animals		9 scorbutic animals		11 insulin treated scorbutic animals	
Liver	Muscle	Liver	Muscle	Liver	Muscle
2.055 ±0.295	0.697 ±0.133	0.391 ±0.083	0.137 ±0.039	1.349 ±0.181	0.350 ±0.057

Insulin treatment of the scorbutic guinea pigs did not significantly alter the fasting blood sugar and the blood sugar values of samples of blood taken 45 and 90 minutes after the feeding of glucose. However, after 150 and 180 minutes there was a marked lowering of the elevated blood sugar in the insulin treated animals although the sugar level was still significantly higher than that of the normal animal. The peak of the blood sugar value of the scorbutic guinea pig was also shifted from the 150th minute period to the 90th minute period after glucose was fed under the action of insulin. In this respect it simulated the glucose tolerance pattern of normal controls.

Glycogen content of the liver and skeletal muscle diminished in scorbutic guinea pigs in comparison with that of the normal controls and the decrease was highly significant statistically. Treatment with insulin strikingly improved the glycogen content of the liver and the skeletal muscle of the scorbutic guinea pigs, though the values did not reach the normal level. It was also observed that the dosage of insulin used in these experiments did not affect the glucose tolerance of normal guinea pigs. These results

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indicated that treatment with insulin for a prolonged period could correct to a great extent the disturbed carbohydrate metabolism in scurvy

At present there are increasing evidences that insulin is involved in the intermediate metabolism of carbohydrates at the level of the Krebs cycle It is possible that in scurvy there may be a disturbance in carbohydrate metabolism at the level of the Krebs cycle due either to diminished insulin production or to the direct effect of lack of vitamin C on the enzyme systems concerned with the oxidation of intermediates through the Krebs cycle The tissue contents of citric, malic and lactic acids were, therefore, determined in normal, scorbutic and insulin treated scorbutic guinea pigs (Banerjee, Biswas and Singh, 1958) The results are given in Table VII, VIII and IX

TABLE VII

Citric acid content of tissues of guinea pigs (mg /100 gm wet tissue)

Animal	Blood	Liver	Kidney	Brain
Normal (12)	5 08±0 30	6 47±0 26	11 27±0 59	7 01±0 61
Scorbutic (12)	7 87±0 17	10 65±0 57	17 96±0 85	12 04±0 75
Insulin treated scorbutic (8)	6 88±0 70	6 18±0 32	13 97±0 92	7 60±0 35

TABLE VIII

Malic acid contents of tissues of guinea pigs (mg /100 gm wet tissue)

Animal	Blood	Liver	Kidney	Brain	Cardiac muscle
Normal (8)	3 42±0 34	20 32±0 88	12 98±0 95	13.43±1 63	16 93±1 91
Scorbutic (8)	8 12±0 80	26 97±2 09	26 60±2 11	25 74±1 67	24 03±1 73
Insulin treated scorbutic (6)	4 51±0 37	17 55±2 20			18 24±1 24

TABLE IX

Lactic acid contents of tissues of guinea pigs (mg /100 gm wet tissue)

Animal	Blood	Liver	Kidney	Brain
Normal(10)	94± 4 5	142±6 9	228±16 0	173± 8 6
Scorbutic (10)	204±15 9	223±8 4	366±17 9	251±11 9
Insulin treated scorbutic (8)	55± 5 0	96±1 7	170±17 7	109± 4 6

Figures in parenthese indicate the number of animals

Table VII shows an increased content of citric acid in the tissues of the scorbutic animals in comparison with that of the normal controls Treatment with insulin brought the level of citric acid to normal This defect in citric acid metabolism may possibly be due to the presence of a metabolic block below the level of citric acid in the Krebs tricarboxylic acid cycle Insulin corrected the defect in citric acid metabolism, possibly by activating the aconitase enzyme and thereby facilitating metabolism of citrate

Malic acid contents of tissues are increased in scorbutic guinea pigs in comparison with those of the normal controls Insulin treatment lowered the level of malic acid to normal in blood, liver and cardiac muscle The increased malic acid content may be related to the increased accumulation of citric acid which is reversibly converted to malic acid through the condensing enzyme-malic dehydrogenase system Malic acid may also be formed by the reaction of carbon dioxide, pyruvic acid and TPNH Pyruvic acid excretion is increased in scurvy (Banerjee and Biswas, 1959) The observation that treatment with insulin lowers the levels of both citric acid and malic acid to normal levels supports the conclusion that lack of insulin is an important factor in the carbohydrate metabolic derangement in scurvy

Table IX shows a greatly increased lactic acid content of the tissues in scorbutic guinea pigs in comparison with that of the normal guinea pig Treatment with insulin lowered lactic acid value even below normal The accumulation of lactic acid may be due to decreased glycogen formation from lactic acid in scurvy

To study further the operation of the tricarboxylic acid cycle in scurvy the urinary excretions of ketone bodies, citric acid and malic acid were determined in normal, scorbutic, insulin treated scorbutic and Fe^{++} and cysteine treated scorbutic guinea pigs after the feeding of butyrate After the collection of a 24 hour sample of urine under toluene each animal was fed daily in three divided doses (Banerjee and Kawishwar, 1959) a neutral solution containing 25 per cent of butyric acid (1 ml/100gm body weight) for 3 consecutive days Urine samples were collected over 24 hours After the above experiments, the animals were rendered scorbutic by the withdrawal of the ascorbic acid supplement. They were divided into three groups Animals of one of the groups were injected with protamine zinc insulin one injection per day, in doses increasing from 0 1 to 0 3 units/100 gm of body weight from the second week of the scorbutic regime To a second group of animals, intraperitoneal injections of a mixture of 0.5 ml. of 0 05 M Fe^{++} as ferrous ammonium sulphate and 0 5 ml. of 0 2 M cysteine hydrochloride were given from the third week of the scorbutic regime The animals of the third group served as untreated scorbutic controls When the animals showed gross signs of scurvy, they were fed butyrate for three days Twenty four hour urine samples, collected before and after the administration of butyrate, were analysed for ketone bodies, citric acid and malic acid The results are given in Tables X and XI

TABLE X

Twenty-four hour urinary excretion of ketone bodies citric acid and malic acid by guinea pigs

Animals	Ketone bodies μ-moles	Citric acid μ-moles	Malic acid μ-moles
Normal (6)	4 87±0.436	14 80± 2 31	3 02±0.26
Insulin-treated normal (4)	2 75±0 014	13 20± 2 10	3 56±0.52
Scorbutic (6)	5 73±0 541	38 87±14.45	4 53±0 903
Insulin-treated scorbutic (6)	4 92±0 208	12 17± 3 15	3 08±0 204
Fe^{++} + cysteine-treated scorbutic (6)	6 10±0.245	15 91± 1.45	1 84±0 342

Figures in parenthes.s indicate the number of animals
Values are mean ± standard error

TABLE XI

Twenty-four-hour urinary excretion of ketone bodies, citric acid, and malic acid by guinea pigs fed sodium butyrate

	μ-moles	μ-moles	μ-moles
Normal (6)	62.64±4.473	46.99± 5.55	8.77±0.904
Insulin-treated normal (4)	10.15±0.975	45.46±11.983	6.44±1.143
Scorbutic (6)	79.39±8.051	438.43±85.03	20.92±1.475
Insulin-treated scorbutic (6)	28.86±3.055	68.36±11.33	4.04±0.544
Fe^{++} + cysteine-treated scorbutic (6)	55.87±13.17	282.10±50.65	6.63±0.198

The urinary excretion of ketone bodies increased enormously in animals of all the groups after they were fed butyrate. The increased urinary excretion of citrate may be due to decreased oxidation of citrate in scurvy. The increased urinary excretion of malic [illegible]

genous insulin possibly promotes increased oxidation of ketone bodies by peripheral tissues, stimulates the production of more oxaloacetate through restoration of the normal operation of the Krebs cycle or stimulates fatty acid synthesis. It seems that insulin released in normal guinea pigs was not sufficient to deal with the excessive amount of ketone bodies formed as a result of the feeding of butyrate.

[illegible] formation of [illegible] rjee, [illegible] and [illegible] to normal after [illegible] seem to indicate [illegible]

In order to see if ascorbic acid has any effect in the treatment of diabetic patients, the effect of administration of vitamin C on the glucose tolerance test and urinary [illegible] Diabetic patients [illegible] nd [illegible] his [illegible] ek the oral glucose tolerance test was performed. The patients were then fed ascorbic acid (10 mg/kg body weight) for three weeks and the glucose tolerance test was repeated. The results are given in Table XII (Banerjee and Ghosh, 1950).

Twenty-four-hour urine samples were collected in these patients, before and during the administration of ascorbic acid for three weeks. The urinary excretions of sugar were determined. In 10 out of 14 cases urine became sugar free three weeks after the administration of ascorbic acid. In order to determine if the diabetic patients were suffering from vitamin C deficiency the 24-hour urinary excretion of ascorbic acid was determined after administration of ascorbic acid (10mg/kg body weight) for 2 to 4 days in 20 diabetic and 5 normal subjects. The results are given in Table XIII.

TABLE XII

Glucose tolerance test in diabetic patients (16 patients)

Treatment	Mg glucose per 100 ml blood				
	0-hour	½-hour	1 hour	1½-hour	2-hours
		after the feeding of 50 gm glucose			
None	143±10	206±13	256±13	271±14	243±16
After feeding ascorbic acid (10mg/kg) for three weeks	113± 5	177± 8	-30±12	231±11	204±11
	2 6*	1 8	1 4	2 2*	2 0*

TABLE XIII

Urinary excretion of ascorbic acid by 10 diabetic and 5 normal subjects

Subjects	Twenty four hour urinary excretion of ascorbic acid in mg				
	Before ascorbic acid	Days after the administration of ascorbic acid			
		1-day	2-days	3-days	4-days
Diabetic	18±3	33± 7	59±21	132+27	178±48
Normal	16±4	288±67	349±92		
	0 6	3 8	3 1		

The fasting blood sugar level of diabetic subjects was significantly lowered after the administration of ascorbic acid The blood sugar values 1½ and 2 hours after the feeding of gl [illegible]

results indicate that diabetic patients suffered from ascorbic acid deficiency The absence of sugar in the urine and the improved sugar tolerance after the diabetic patients were fed ascorbic acid for three weeks indicated that ascorbic acid led to the increased production of insulin in these patients

TABLE XI

Twenty-four-hour urinary excretion of ketone bodies, citric acid, and malic acid by guinea pigs fed sodium butyrate

	μ moles	μ-moles	μ-moles
Normal (6)	62 64±4.473	46 99± 5 55	8 77±0 904
Insulin-treated normal (4)	10 15±0 975	45 46±11 983	6.44±1 143
Scorbutic (6)	79 39±8 051	438.43±85 03	20 92±1.475
Insulin treated scorbutic (6)	28 86±3.055	68 36±11 33	4 04±0 544
Fe^{++} + cysteine-treated scorbutic (6)	55 87±13 17	282 10±50 65	6 63±0 198

The urinary excretion of ketone bodies increased enormously in animals of all the groups after they were fed butyrate The increased urinary excretion of citrate may be due to decreased oxidation of citrate in scurvy The increased urinary excretion of malic

bodies was distinctly lowered by the insulin treatment of the scorbutic animals Exogenous insulin possibly promotes increased oxidation of ketone bodies by peripheral

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lactic dehydrogenases in scurvy The activity of the enzymes was restored to normal after prolonged treatment of the animals with insulin All these observations seem to indicate that vitamin C-deficiency leads to hypoinsulinism

In order to see if ascorbic acid has any effect in the treatment of diabetic patients the effect of administration of vitamin C on the glucose tolerance test and urinary

diet during their stay in the hospital After the patients received this diet for one week the oral glucose tolerance test was performed The patients were then fed ascorbic acid (10 mg /kg body weight) for three weeks and the glucose tolerance test was repeated The results are given in Table XII (Banerjee and Ghosh, 1950)

Twenty-four-hour urine samples were collected in these patients, before and during the administration of ascorbic acid for three weeks The urinary excretions of sugar were determined In 10 out of 14 cases urine became sugar free three weeks after the administration of ascorbic acid In order to determine if the diabetic patients were suffering from vitamin C deficiency the 24-hour urinary excretion of ascorbic acid was determined after administration of ascorbic acid (10mg /kg body weight) for 2 to 4 days in 20 diabetic and 5 normal subjects The results are given in Table XIII

TABLE XII

Glucose tolerance test in diabetic patients (16 patients)

Treatment	Mg glucose per 100 ml blood				
	0-hour	½-hour	1-hour	1½-hour	2-hours
		after the feeding of 50 gm glucose			
None	143±10	206±13	256±13	271±14	243±16
After feeding ascorbic acid (10mg/kg) for three weeks	113± 5	177± 8	-30±12	231±11	204±11
	2 6*	1 8	1 4	2 2*	2 0*

TABLE XIII

Urinary excretion of ascorbic acid by 20 diabetic and 5 normal subjects

Subjects	Twenty four-hour urinary excretion of ascorbic acid in mg				
	Before ascorbic acid	Days after the administration of ascorbic acid			
		1-day	2-days	3-days	4-days
Diabetic	18±3	33± 7	59±21	132±27	178±48
Normal	16±4	288±67	349±92		
	0 6	3 8	3 1		

The fasting blood sugar level of diabetic subjects was significantly lowered after the administration of ascorbic acid The blood sugar values 1½ and 2 hours after the feeding of glucose were significantly lower when the patients received ascorbic acid for three weeks This indicated that the utilization of glucose was slightly improved when the diabetic patients were given ascorbic acid The normal subjects excreted significantly increased amounts of ascorbic acid than diabetic patients, when fed ascorbic acid The results indicate that diabetic patients suffered from ascorbic acid deficiency The absence of sugar in the urine and the improved sugar tolerance after the diabetic patients were fed ascorbic acid for three weeks indicated that ascorbic acid led to the increased production of insulin in these patients

It was of interest to find out how insulin production might be influenced by vitamin C-nutrition of the body Insulin contains a large amount of cystine and it is probable that under the influence of enzymes cystine is taken up by beta cells to form the insulin molecule In the body cystine may be derived either from methionine or cysteine, and glutathione the naturally occurring tripeptide, contains cysteine If the glutathione content of the body is diminished, it may interfere with the normal synthesis of insulin Certain enzymes of the body depend on -SH groups for their activity, and if the -SH groups [illegible] The activity, however, may be [illegible] Glutathione thus is supposed to [illegible] glutathione content of tissues is [illegible], the beta cell sulfhydryl enzymes, necessary for the synthesis of insulin, may not be well protected from the toxic action of unknown substances leading to the hypofunction and diminished secretion of insulin Intravenous injection of dehydroascorbic acid in rats leads to chronic hyperglycemia and glycosuria Glutathione if injected

significantly Cholesterol content of the different tissues varied differently in the scorbutic animals, it diminished in the adrenals spleen, and lungs considerably during scurvy This may be due either to diminished synthesis by these tissues or to reduced transport The increase in the cholesterol content of the testes during scurvy might be associated [illegible]

production Cholesterol content of intestine increased and that of liver or kidney did not show any change during scurvy From this study it could not be ascertained if the lower adrenal cholesterol in scurvy was due to diminished synthesis of cholesterol or increased production of adrenal cortical hormone

[illegible]

pigs (Banerjee and Singh 1958*a*) The results are given in Table XIX

TABLE XIX

Total body cholesterol content of normal scorbutic, and insulin treated scorbutic guinea pig (mg per 100 gm wet tissue) No of animals - 6

Total cholesterol content			*t* values		
Normal	Scorbutic	Insulin treated scorbutic	Between normal and scorbutic	Between normal and insulin treated scorbutic	Between scorbutic and insulin treated scorbutic
180±14	277±27	173±15	2 0	0 7	3 0

The total body cholesterol content of scorbutic guinea pigs increased significantly in comparison to that of normal controls This possibly indicates increased synthesis of cholesterol in scurvy The decreased cholesterol of adrenals in scorbutic guinea pigs therefore might be due to increased formation of adrenocortical steroids Insulin treatment lowered the body cholesterol of scorbutic guinea pigs to normal level The increased cholesterologenesis in scurvy therefore might be due to the utilization of an increased pool of acetate which is not burned through tricarboxylic cycle in scurvy

Vitamin C-deficiency results in enlargement of adrenal glands (Banerjee and Ghosh 1946) A decrease in liver and muscle hexokinase activity in scorbutic guinea pigs has also been reported (Banerjee and Ghosh 1955) Adrenal cholesterol is diminished in scurvy All these investigations indicated deranged activity of the adrenal cortex in scurvy The urinary excretions of 17-ketosteroids and corticosteroids were therefore determined during the progress of scurvy to determine the adrenocortical activity Guinea pigs and monkeys were used as the experimental animals After determining the normal excretions ascorbic acid supplement was withdrawn and these excretions were estimated during the different weeks after withdrawal of ascorbic acid The results are given in Tables XX XXI and XXII (Banerjee and Singh 1957)

TABLE XX

Twenty-four hou r uri ary excret on of 17-keto tero ds by fema e g nea p gs
(g es reted a al day)

Animal No	Before withdrawal of ascorbic acid	Weeks after withdrawal of ascorbic acid: 1	2	3	3½	4
1	135	26	64	117	159	Died
2	89	60	88	110	307	Died
3	96	56	1	117	376	Died
4	105	83	78	202	Died	
8	168	502	33	245	115	Died
11	83	57	67	96	Died	
14	164	154	84	71	87	125
9	76	94	57	45	43	Died
12	88	80	85	70	47	31
13	129	138	110	123	4	Died

TABLE XXI

Twenty-four hour uri ary ex ret on of 17 ket ro ds by fe na e mon y
(μg excre ted an al day)

Monkey No	Before withdrawal of ascorbic acid	Months after withdrawal of ascorbic acid: ½	1	1½	[illegible]	½	3	3½	4
1	808	685	682	6 7	511	630	767	959	3003
2	644	547	671	600	42	3 4	662	2712	Died
3	712	555	813	361	609	54	598	780	1047

TABLE XXII

Twenty-four-hour ur ry excret o of 17 keto tero ds (17 KS) and orti o tero ds (CS) by female gu nea p g (μg ex retion an a day

Pair No		Weeks after withdrawal of ascorbic acid: 1, 17-KS	1, CS	2, 17 KS	2, CS	2½, 17 KS	2½, CS	3½, 17 KS	3½, CS
1	Normal	225	238	217	162	127	81	222	258
	Scorbutic	22	175	250	187	11	118	416	363
2	Normal	166	160	139	207	1 7	200		
	Scorbutic	160	140	209	243	571	375	Died	
3	Normal	371	262	2 8	197	370	248	400	
	Scorbutic	-22	100	375	257	391	247	1 5	591
4	Normal	277	175	250	292		287		
	Scorbutic	118	288	333	392		481	Died	
5	Normal	200	280	165	362	119	231		-18
	Scorbutic	298	417	324	405	347	287		521

It was observed that the excretion of 17-ketosteroids diminished in the first week the animals were on the scorbutic diet, then the excretion was irregular and when the animals became acutely scorbutic, 7 out of 10 guinea pigs and 3 monkeys excreted larger amounts of 17-ketosteroids The remaining three severely scorbutic guinea pigs however showed a diminished urinary excretion of 17-ketosteroids

When scurvy is developed in the animals, food consumption is diminished and inanition itself might alter adrenocortical function Excretion patterns of both 17 ketosteroids and corticosteroids were, therefore, studied in guinea pigs during the process of development of vitamin C-deficiency and in paired fed control animals While the excretion of these steroids did not vary considerably in the control animals during the experimental period, four out of five severely scorbutic guinea pigs excreted increased amounts of both the steroids which indicated hyperfunction of the adrenal cortex in those animals during the later part of the experimental period One of the severely scorbutic animals, however excreted diminished amounts of both these steroids

The results obtained prompted to conclude that the levels of steroids of adrenocorti cal origin in urine r [illegible] of the body It is possible [illegible] be responsible for [illegible]

Thyroid activity in normal and scorbutic guinea pigs

[illegible]

Guinea pig [illegible] scorbutogenic diet only (b) the scorbutc [illegible] diet with 0 05% desi [illegible] mg ascorbic acid pe [illegible] aily supplement of [illegible] mm C The oral glucose tolerance test was performed on the 22nd day in all the animals The results are given in Table XXIII

TABLE XXIII

Glucose tolerance test in guinea pigs on different diets

Animal	Fasting blood sugar (mg %)	Blood sugar after feeding glucose (mg%)			
		45 min	90 min	135 min	180 min
Scorbutic (8)	150± 2 2	326±23 9	333±22 4	306±31 1	251±37 1
Scorbutic with methylthiouracil (8)	151±10 7	298±23 5	316±31.2	333±24 4	266±37.2
Scorbutic with thyroid (8)	151± 5 3	331± 3 4	317±17 0	251±17.2	210±16 4
Normal (8)	132± 5.4	279±11 7	251±17 2	194±14 9	136± 4 1
Normal with methylthiouracil (7)	121± 5 7	261±10 8	256± 8 2	196±12 6	146±14 9
Normal with thyroid (8)	147± 5 5	271±10 5	262±17 3	210±20 2	170±33 9

Figures in parenthesis indicate the number of animals

There was not much differences in the glucose tolerance test in the animals of the different groups The altered carbohydrate metabolism observed in scorbutic guinea pigs, therefore, may not be due to either hypo or hyperthyroidism. These observations indicate that thyroid function may not be disturbed in scurvy

Structural changes in testes in scorbutic guinea pigs

Chronic scurvy was produced in guinea pigs by feeding them a scorbutogenic diet long with a daily oral supplement of 0 25 mg of ascorbic acid Histological studies ere carried out in normal, scorbutic and chronically produced scorbutic guinea pigs (Mukherjee and Banerjee, 1954) Complete degeneration of Leydig's cells with increased fibrosis and coagulative necrosis of the seminiferous tubules were observed in chronic scurvy In acute scurvy the Leydig's cells only showed early degenerative changes but seminiferous tubules were normal The degenerative changes of Leydig's cells in the testes of scorbutic guinea pigs indicate hypofunction of testes The observation of increased cholesterol content of the testes of scorbutic guinea pigs (Belavady and Banerjee, 1954) indicated that possibly cholesterol could not be utilised by the degenerated Leydig's cells for the synthesis of the testicular hormone The absence of degenerative changes in the varies of scorbutic guinea pigs indicated that the gonadotrophic hormone of the anterior ituitary was not involved in the degenerative changes observed in the testes of scorbutic uinea pigs It was not possible to study the function of the pituitary gland in scorbutic uinea pigs as it had not been possible to remove the pituitary gland from guinea pigs

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ACTION OF DRUGS ON THE RELEASE OF OXYTOCIN IN UNANAESTHETIZED RABBITS

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ABSTRACT

Alcohol and water which cause a block in the release of ADH also cause a block in the release of oxytocin This pharmacological tool can be made use of to investigate if sperm migration at coitus is dependent on the release of oxytocin The results also confirm earlier work that simultaneous release or block of the neurohypophysial hormones always occurs Chlorpromazine caused a block in the release of oxytocin Oestrogen in doses which blocked the anterior pituitary and reserpine in the doses used caused no block in the release of oxytocin

It is generally believed that uterine contractions presumably caused by oxytocin

tigated

EXPERIMENTAL PROCEDURE

Lactating rabbits 2 5 to 3 kgm in weight were taken 10-12 days after birth of the ... the pups

weight after suckling of the pups after separation overnight as modified ... (1956) When the pups gained the same weight after feeding on three consecutive mornings the drug was administered to the mother prior to feeding on the next day If the pups did not gain weight after suckling for fifteen minutes then an intravenous injection of oxytocin (20-100 milli units) was made into the marginal ear vein of the rabbit and the pups allowed to suckle again When the pups increased in weight after this second feeding then only was it concluded that the release of oxytocin had specifically been blocked by the administered drug The percentage block has been calculated ... weight on previous

Oxytocin was administered in the marginal ear vein Chlorpromazine was administered immediately before feeding by intravenous injection in the ear vein of the animal.

Oestrogen and reserpine were administered intraperitoneally at various intervals before feeding of the pups

Alcohol and water were administered by means of a soft rubber catheter 30-40 minutes before feeding

Synotocinon (synthetic oxytocin, Sandoz Products) was used in all experiments Ovocyclin P(oestradiol dipropionate Ciba Ltd) serpasil (Reserpine Ciba Ltd) and Largactil (chlorpromazine May and Baker Ltd) were the substances used

RESULTS

[illegible] 10 per [illegible] before [illegible] the block was reversed by intravenous injection of oxytocin (20-100 milliunits)

TABLE I

Blockage of Milk Ejection in Rabbit

Drug	Litter B	Litter C	Litter D	Litter E	Litter G	Litter H	Litter I
Alcohol (7 5% 10% orally)	Blocked 100%	Blocked 100%	Blocked 100%	Blocked 100%	Blocked 85%	Blocked 100%	—
Chlorpromazine (5mgm/kgm I P)	Blocked 88%	Blocked 74%	Blocked 100%	Blocked 95%	No Block	No Block	Blocked 100%
Reserpine (2 5 mgm-6 mgm kgm I P)	No Block	No Block	No Block	No Block	—	No Block	No Block
Oestrogen (100-200 Microgram I.P)	No Block	No Block	No Block	No Block	—	No Block	—
Water (5 ml/100 gm Body weight)	—	—	—	Blocked 100%	Blocked 80%	Blocked 100%	Blocked 100%

Chlorpromazine administered in the dose of 5 mgm /kgm body weight intravenously immediately befor [illegible] seven animals In those anim [illegible] the dose of chlorpromazine [illegible]

Reserpine was administered to six animals intraperitoneally (1 5-4 0 mgm /kgm body weight) at intervals of 1 hour 2 hours and 3½ hours before allowing the pups to suckle In none of these animals was the suckling reflex abolished When the dose of reserpine was further increased it proved to be toxic to the animals which died the next day In these cases the milk ejection reflex was abolished but the blocking action of reserpine on the suckling reflex was not reversed by intravenous injection of oxytocin

Oestrogen was administered in doses 150-200 micrograms intraperitoneally 2-3½ hours before suckling In none of the five animals to whom it was administered did it cause a block of the milk ejection reflex

Water—In four experiments the action of hydration on the milk ejection reflex was investigated It was found that when tap water (5 ml/100 gms body weight) was

administered to lactating rabbits 30-40 minutes prior so suckling the milk ejection reflex was abolished and this block could be reversed by intravenous administration of oxytocin

Discussion

It has been shown by Abrahams and Pickford (1954) in bitches and Harris (1955) in rabbits that whenever one of the neurohypophysial hormones is released the other is also released These observations based on the measurement of uterine motility and antidiuresis have been confirmed by direct measurement of the hormones in blood after stimulation of the neurohypophysis by drugs (Chaudhury and Walker 1958) Since the hormones are always released simultaneously it is not surprising that alcohol and water which are known to block the release of ADH also block the release of oxytocin as has been demonstrated in these experiments This may be used as a pharmacological tool

now be in-
cker (1959)
days after

puerperium the uterine contractions were not in any way affected by hydration of the subject He argues that a differential release of the two hormones is therefore possible. It is contended that uterine contractions as observed by Dicker are not a specific criterion for assessing the release of oxytocin as the exact role of oxytocin in parturition is not yet clear Milk ejection is a more specific action of oxytocin and in the above experiments on the rabbit it has very definitely been blocked by alcohol and water and then reversed by adminstration of intravenous oxytocin The above results go against the possibility

caused no block at all in two of the animals

be added that Moon and Turner (1959) have recently shown that Reserpine inhibits the release of oxytocin as judged by milk-let down yield per time nursing in rats *

mammary glands and even Oxytocin injections could not eject out any milk. The oestrogen had it appeared completely blocked the secretion of milk.

Acknowledgements

He would like to acknowledge the willing and efficient technical assistance of Mr T C Joseph

* Added by the Convener

REFERENCES

THYROID STATUS AND ADRENAL CORTICAL ACTIVITY

by J S RAWAT and A ROY

U P College of Veterinary Science and Animal Husbandry, Mathura

corte

corti...

gross pathogenesis of exophthalmic goiter partly on the basis of under functioning of the adrenal cortex Certain manifestations are common to over function of the thyroid and under function of the adrenal, Zondek (1935) has stated that thyroidectomized animals survive total adrenalectomy longer than those possessing thyroids Koelsche and Kendall (1935) have found that adrenocortical hormones exert a sparing action against the negative nitrogen balance induced by thyroxine

A systematic study was undertaken to elucidate the changes that take place in the adrenal cortex physiology in hypothyr...

±10 gms , and age 90 0±7 days were

15 mg thiourea mixed in their food

periods and thus different stages of

of animals was maintained as control and this animal was given same amount of food as consumed by the respective thiourea fed animal Animals in the various other groups received alongwith thiourea, thyroxine administered orally and/or ACTH subcutaneously The dosage of the hormone was 5γ of 1-thyroxine sodium and 0 2 IU ACTH daily

... killed following anaesthetization ... ministered intraperitoneally The ... fore slaughter When completely anaesthetized the adrenals were dissected out and weighed in a torsion balance and moisture and cholesterol was estimated in the left adrenal gland and ascorbic acid in the right Cholesterol and ascorbic acid were estimated as described by King (1947)

The results obtained from animals of various groups are given in Tables 1 to 8 and the observations are discussed below —

WEIGHT OF ADRENAL

Weight of adrenal glands at weekly intervals during the progress of hypothyroidism as a result of thiourea feeding and also during its regress by stopping thiourea administration after feeding it for 28 and 42 days was noted

... thyroid hypertrophy, ... in weight on the third

During the post thiourea feeding period the adrenal glands were gaining in weight but the degree of improvement in those where thiourea was administered for 6 weeks, was less marked, it appears that prolonged hypothyroidism results in more lasting injury to the adrenals (Table 2)

The atroph ... during this ... hence ACTH ... influence of t ... adm ...

... ration of thyroxine and ... esponsive to ACTH administration ... it show... hand cause slight hypertrophy of the adrenals. The implication of these observations are discussed in detail later

CHOLESTEROL

Cholesterol content of the ... condition for so long a per... weight of tissue matter or ... content ... cho... weig... tration of thyrox... acid content of t... untreated pair fe... may, therefore, b... ACTH ...

cortical horr... and Rosen a... was administ... more than th... were capable o... ACTH during I... terol I... cortex... due t... tered... size a... nal co... ... sync... It is not clearly known what fact... of cholesterol We offe... other factors is depen... content of pituitary is ... in our experiments... is decreased. In or... with thiourea not o... laden with choleste... cholesterol which rem... pr... also account for the

TABLE 5

Composition of adrenals of animals during regress of hypothyroidism maintained for 4 weeks

Days		Absolute content (mg)			per 100 mg of adrenal in mg		
		Moisture	Ascorbic acid	Choles-terol	Moisture	Ascorbic acid	Choles-terol
7	TU Fed	6 8	020	48	71	221	5 0
		± 242	± 0003	± 022	± 2 5	± 003	± 23
	PFC	7 0	039	80	72	396	8 0
		± 200	± 0004	± 054	± 2 0	± 004	± 53
			× × ×	× × ×		× × ×	× × ×
14	TU Fed	7 2	036	75	72	377	7 5
		± 204	± 0004	± 048	± 2 0	± 004	± 48
	PFC	8 0	041	88	71	394	7 8
		± 220	± 0004	± 054	± 1 9	± 004	± 48
21	TU Fed	7 6	039	83	72	354	7 8
		± 263	± 0003	± 037	± 2 5	± 003	± 35
	PFC	7 9	044	91	72	402	8 3
		± 229	± 0004	± 044	± 2 1	± 004	± 40
28	TU Fed	8 8	040	80	74	359	6 7
		± 362	± 0005	± 072	± 3 0	± 004	± 60
	PFC	9 4	047	80	73	407	6 0
		± 369	± 0005	± 044	± 2 8	± 004	± 34

TABLE 6

Composition of adrenals of animals during regress of hypothyroidism maintained for 6 weeks

Days		Absolute content (mg)			Per 100 mg of adrenal in mg		
		Moisture	Ascorbic acid	Choles-terol	Moisture	Ascorbic acid	Choles-terol
7	TU Fed	7 6	022	68	72	241	7 6
		± 283	± 0002	± 043	± 3 1	± 002	± 48
	PFC	8 1	039	81	72	356	7 4
		± 31	± 0003	± 063	± 2 8	± 002	± 57
			× × ×	>		×	
14	TU Fed	6 8	033	68	71	383	4 7
		± 312	± 0003	± 040	± 3 0	± 004	± 42
	PFC	7 7	047	68	69	443	5 6
		± 189	± 0003	± 034	± 2 2	± 003	± 30
			× × ×			× × ×	
21	TU Fed	6 9	023	65	69	254	6 6
		± 248	± 0002	± 031	± 2 5	± 002	± 51
	PFC	8 4	038	68	70	332	5 6
		± 252	± 0003	± 042	± 3 0	± 002	± 35
			× × ×			× × ×	

TABLE 7

Composition of adrenals as affected by simultaneous administration of thiourea and Hormones, continuously for 28 days

(For ready reference the composition of adrenals of 28 day thiourea fed animals and their pair fed controls have also been incorporated in the table)

	Absolute content in mg				
	Pair fed control	Thiourea	Thiourea TX	Thiourea, ACTH	Thiourea, TX, ACTH
Moisture	7 1 ± 220	6 90 ± 211	7 0 ± 240	6 9 ± 314	7 3 ± 214
Ascorbic acid	0 270 ± 0003	0 018 ± 0002	026 ± 0003	020 ± 0002	.020 ±.0002
Cholesterol	0 914 ± 062	0 524 ± 038	900 ±.071	500 ±.041	515 ±.061

TABLE 8

Glycogen content in mg of whole fresh liver of animals as affected by simultaneous administration of thiourea and hormones continuously for 28 days

(For ready reference the composition of liver of 28 day thiourea fed animals and their pair fed controls have also been incorporated in the table)

	Pair fed control	Thiourea	Thiourea TX	Thiourea ACTH	Thiourea, TX, ACTH
Glycogen as glucose	40 ±4	120 ±28	50 ±12	125 ±30	40 ±8

DISCUSSION

Subrahmanian Dr Banerjee, what is the effect of vitamin C-deficiency on protein metabolism?

Different fractions of plasma proteins were determined by paper electrophoresis in

genesis is not clear

Prasad Dr Banerjee, what is the relationship between decrease in hexokinase activity and ascorbic acid deficiency?

there was [illegible] decrease in liver hexokinase activity and 8% decrease

very interesting Could

any observation on the

ovaries?

Banerjee No histological changes were observed in the ovaries of scorbutic guinea pigs *It is, therefore,* unlikely that the degenerative changes in the testes was of pituitary origin due to defect in the

It seems that ascorbic acid which plays a role in the

testicular

resulting

in its degeneration

Das Gupta Dr Banerjee, what was the level of ascorbic acid in the adrenals of scorbutic guinea

compared to normal?

00 gm

ormone

s and as

cretion

bserved

se these

SECTION D

PITUITARY—ADRENOCORTICAL MECHAMSMS

STUDIES IN ENDOCRINAL INTERRELATIONSHIP USING A HISTOCHEMICAL METHOD FOR THE EVALUATION OF SUCCINIC DEHYDROGENASE ACTIVITY AS AN INDEX

by E J de Souza and T H Rindani

Department of Anatomy and Physiology Topiwala National Medical College, Bombay 8

Abstract

Interrelated activity between the thyroid and testis, the thyroid and the adrenal cortex, and between the adrenal cortex and the testis has been histochemically demonstrated in experimentally treated rats

Alterations in thyroid status affect both the levels and activity patterns in the testes and supra renal cortex

Compensatory atrophy of the adrenal cortex while also showing changes in level and pattern of S D activity in itself also brings about general lowered activity in the testis as well as interfering with the activity level in spermatogenesis

Both thyroidectomy and compensatory adrenocortical atrophy lowered the activity of S. D in the liver as well as altered the zonal pattern

The functional interrelationship among the various endocrines is well known That the activity of the gonads is influenced by the adenohypophysis the adrenal cortex and the thyroid has been shown both in experimental animals as well as in clinical syndromes associated with the anomalies of these glands

The methods used so far for demonstration of these interrelationships have been mostly clinical evidences of altered function or morphological and biochemical changes in experimental animals (Rindani 1957 1958)

The application of histochemical methods to the study of the biological activity of important cellular elements of various organs in the body has however the distinct advantage of locating sites of altered activity under various conditions Thus the localization of succinic dehydrogenase is of great value in this respect as the specificity of the enzyme -substrate reaction and the demonstration of its activity by means of a tetrazolium (Nitro B T) reduction technique renders it as one of the ideal indices of organ metabolism at a histochemical and even cytochemical level (De Souza and Kothare 1959)

Succinic dehydrogenase activity has been shown to be altered in the liver tissues under conditions of change in gonadal function (Rindani 1958) Even the highly specialized structure like the spermatozoon lends itself to such a method of evaluation of the physiological normality or otherwise (Kothare and de Souza 1958)

This study was therefore undertaken to investigate the thyroidgonadal relationship by noting the variations from normal in the succinic dehydrogenase activity of the testis under altered conditions of thyroid activity Similarly the same enzyme was used to note the relationship between thyroid status and adrenocortical activity and the influence of the adrenal cortex on the gonads

Materials and Methods

The studies were carried out on male albino rats weighing about 100 g each The animals were divided into four groups and treated as under —

The basis of this technique lies in the fact that whenever the enzyme succinic dehydrogenase is active, electrons are released during the breakdown of succinic acid to fumaric acid The electrons are normally utilised in the reduction of the cytochromes and further made available for the synthesis of ATP and protein or for energy requirements of cells in their burst of metabolic, motor or secretomotor activities

By the use of tetrazolium salts in general and p Nitrophenyl substituted ditetrazole (Nitro BT) in particular these electrons reduce the normally soluble protein binding colourless tetrazolium into a blue insoluble diformazan at the exact site of enzyme activity (Nachlas *et al* 1957) Nitro BT has special advantages in a study such as this The first is the extremely finely particulate deposition of diformazan rendering it cytochemically an excellent index of the degree of activity in the various layers of the seminiferous tubules and hence in the various stages of spermatogenesis

Another advantage is the fact that the reaction resulting in an alcohol-xylol fast diformazan has great speed and is a high electron competitor, minimizing falsification by a possible cytocheomic alteration and the staining result is thus more truly that of succinic dehydrogenase than is possible with other tetrazolium salts Incidentally this makes possible permanent sections mounted in balsam (one of us E J S has sections four years old without appreciable loss of staining results)

Further, the fact that a fat soluble monoformazan is at a minimum is important in organs with high fat content like the adrenal (especially during adrenal hypoplasia) as diffusion artefacts and false cytological localization are prevented This is best exemplified in the increased fatty deposition that takes place in a broad belt in the peripheral portion of the zona fasiculata and the glomerulosa in induced hypoplasia of the adrenal This is seen as a belt of almost total inactivity This is quite different from normal fat cells where even though the fat is negatively stained the cytoplasm of normal adipose tissue is quite heavily loaded with formazan

The method besides classifying zonal patterns of enzyme activity and intracytoplasmic localization can also be used for a rough quantitative estimation of activity as differences in colour intensity denoted differences in activity levels when sections of fairly identical thickness were incubated under uniform conditions for equal times

As the adrenal hypoplasia had to be effected by the administration of hydrocortison acetate the animals were sacrificed after the second day to ensure best effect with no res dual cortison action This was checked histologically by the complete band of fat change in the distal fasculate zone the smaller adrenal weight and by the fact that t lowered SD activity in the check liver sections is in consonance with the findings of Mal and Bourne in adrenalectomised animals (Malaty and Bourne, 1953)

The studies reported here indicate that the hormones of the thyroid and adrenal cortex influence the activity of the testicular function at a metabolic level Where the adrenocortical hormones seem to be concerned with a regulation of the general metabolic activity of all the cells of the seminiferous tubules, the thyroid hormone appears to have an effect on the processes involved in the differentiation of these highly specialised cells

Similarly the adrenocortical activity also seems to be influenced by the thyroid hormone acting at a metabolic level, being probably concerned in the intermediate chemical changes concerned in the synthesis of the cortical hormones So far as the general metabolic effects of the thyroid and adrenal cortex are concerned it seems quite certain that as judged by the influence of the deficiency of their endocrines on the S D activity



Fig 6 Photomicrograph showing S D activity in the normal rat adrenal (x 100)



Fig 7 Photomicrograph showing S D activity in the r adrenal after thyroidectom There is a generally lower activity The high activity is r duced to a few cells deep or in the juxta medullary re (x 100)

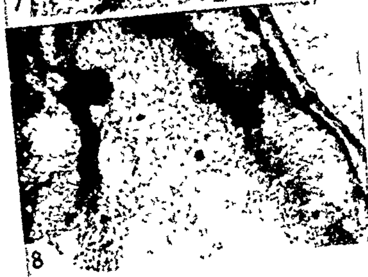


Fig 8 Photomicrograph showing S D activity in the rat adrenal after compensatory atrophy There is lowered activity with almost total absence in the fasciculata but fairly high activity in the glomerulosa (x 100)

in the liver the chemical reactions occurring in liver round the Krebs citric acid cycle are substantially influenced by these hormones

Acknowledgements

The Authors are thankful to Dr L Monteiro Dean, T N Medical College for the interest taken in the studies and to Mr N M Chavan for the assistance rendered in the work.

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INFLUENCE OF ADRENALS ON THE CYTOLOGY AND HISTOLOGY OF GASTRIC MUCOSA AND THE GASTRIC SECRETION OF PYLORUS OBSTRUCTED RATS

by G B Singh and S H Zaidi

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ABSTRACT

Studies on the influence of adrenals on gastric mucosa and the gastric secretion in normal and pylorus obstructed rats have been made

There was no ulceration of the gastric mucosa in normal or adrenalectomized rats without pyloric obstruction Pyloric obstruction in adrenal intact animals caused various grades of ulceration in the forestomach Adrenalectomy completely protected the gastric mucosa from ulceration in pylorus obstructed rats and also significantly reduced the free acidity peptic activity and total volume of gastric juice

Pepsinogen contents of the glandular mucosa was diminished after adrenalectomy in normal rats and could not be detected when adrenalectomy was associated with obstruction

The influence of adrenals on the gastric mucosa of pylorus obstructed rats has been investigated and various views regarding its significance expressed Adrenals played

on gastric mucosa and the gastric secretion in pylorus obstructed rats

Material and Method

Histopathological technique—Autopsy was performed on all the animals sacrificed After opening the abdominal cavity the stomach was inspected in situ for distension, congestion and perforation It was then taken out, a small slit made in the duodenum along the greater curvature and the contents drained in a beaker The stomach was then opened, inspected for any ulceration and graded by the method of Barrett *et al* (1953) The tissue blocks from different portions of stomach were fixed in 10 per cent formal saline and Regaud's fluid After complete fixation the blocks were embedded in paraffin and section cut at 5 μ Sections were stained with haematoxylin and eosin, PAS for mucin (Pearse, 1949) and for pepsinogen by Bensley's neutral gentian stain (Cowdry, 1948)

Biochemical technique—The gastric contents from each animal were centrifuged to remove food particles Peptic activity of gastric juice was estimated as described by Glick (1955) using phenol reagent and casein substrate The results have been expressed as 'pepsin unit' Free and total acidity was titrated with 0 01 N-NaOH using Topfer's reagent and phenolphthalein as indicators and expressed as m eq /L.

Plan Of Experiment

The following groups were constituted —

Group I — Six normal rats

Group II — Six adrenalectomized rats

Group III — Ten non-adrenalectomized pylorus obstructed rats

Group IV — Ten adrenalectomized and pylorus obstructed rats

Experimental Procedure

All the animals were fasted for forty-eight hours Pyloric obstruction was done in groups III and IV by the procedure previously stated and animals sacrificed sixteen hours after the obstruction Rats of groups I and II were further fasted for sixteen hours and sacrificed along with the pylorus obstructed rats This was done because the fasting period of forty-eight hours before the pyloric obstruction followed by sixteen hours when the animals were sacrificed brought about maximum structural changes in the gastric mucosa

Results

Gross appearance—The naked-eye examination of the stomach in situ in groups I (Normal control) and II (adrenalectomized) showed smooth shiny serous layer On opening the stomach was seen to have normal mucous layer Group III (pylorus obstructed without adrenalectomy) showed marked distension congestion of the serous layer On opening the stomach the ulcers were seen to be confined mostly in the forestomach Small ulcers appeared as tiny rounded lesions while the larger ones were irregular or acicular in shape One animal showed ulceration measuring 7 mm one had ulcers 4-6 mm three showed large number of ulcers 1-3 mm and in the remaining three animals few small ulcers measuring 1-3 mm in one diameter were present Two animals died in the process of investigation, the cause of death could not be ascertained Group IV (adrenalectomized and pylorus obstructed) showed slight congestion and distension of the serous layer No ulceration was present in this group of animals Three animals died in the course of experiments The gastric mucosa showed greyish brown appearance The gastric contents were dirty brown in colour (Table I)

Table I

Summary of results of biochemical investigations of gastric juice and degree of ulceration in adrenal intact and adrenalectomized pylorus obstructed rats

Group and animal number		Volume	Acidity Free	Acidity Total	Peptic activity	Degree of ulceration
		c c	m eq L	m eq L	P U * X10*	
Group III (Adrenal intact)	1	5.3	30	100	0.39	1
	2	5.6	40	100	0.40	2
	3	5.5	48	96	0.41	4
	4	5.0	54	108	0.44	3
	5	5.0	74	80	0.43	3
	6	5.1	22	63	0.46	4
	7	4.7	40	56	0.41	3
	8	5.5	45	56	0.37	1

P U *—Peptic Unit.

TABLE 1 (*Concld*)

Group and animal number		Volume	Acidity Free	Acidity Total	Peptic activity	Degree of ulceration
		c c	m eq /L		P U *X10³	
Mean		5 2	44 2	83 0	0 42	2 6
Median		5 2	43 0	88 0	0 41	3 0
Range		4 7—5 5	22 0—74 0	56 0—108 0	0 37—0 46	1 0—4.0
Group IV (Adrenalectomized)	1	3 0	0	24	0 20	0
	2	1.0	22	46	0 10	0
	3	1 5	6	22	0 17	0
	4	0 8	—	—	0 23	0
	5	0 5	—	—	0.20	0
	6	1 8	—	—	0.20	0
	7	1 0	16	24	0 13	0
Mean		1 3	8 8	27.2	0 18	0 0
Median		1 0	6 0	24 0	0 20	0 0
Range		0 5—3 0	0 0—22 0	20 0—46 0	0 10—0 23	0 0

Groups I and II—Sufficient quantity of gastric juice was not available for biochemical investigations

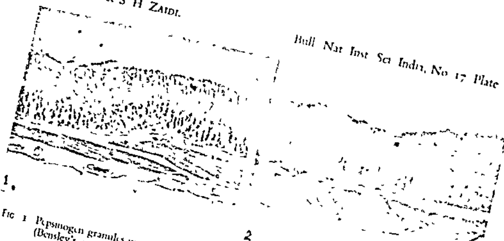
The mean values of gastric juice, free acidity and peptic activity for group III are significantly higher than for group IV at 95 per cent level.

granules in all the animals In group IV, ulceration was not present. Mucosa showed normal appearance except congestion in the submucosal layer Peptic cells in all the animals were mostly devoid of pepsinogen granules (Fig 4)

Mucous cells were not effected in any of the groups Parietal cells were slightly diminished in size in groups III and IV

Biochemical

group IV at 95 per cent level



1

FIG 1 Pepsinogen granules in normal stomach (Bensley's neutral gentral stain X 40

2

granules in normal adre- stomach Bensley's neu- X 4



3

FIG. 3 Early erosion showing destruction of the superficial layer of the squamous epithe- lium H and E X 40

4

FIG 4 Absence of Pepsinogen granules from peptic cells Bensley's neutral gentral stain X 4

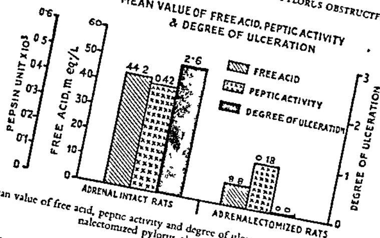


FIG. 5 Mean value of free acid, peptic activity and degree of ulceration in adrenal intact and adrenalectomized pylorus obstructed rats

Acidity—The mean values and the range of free and total acidity are summarised in Table I and Fig 5 Enough gastric juice was not available for biochemical estimation of free and total acidity in groups I and II The mean value of free acidity in group III was 41 2 m eq /L and in group IV only 8 8 m eq /L The mean value of free acidity in group III is significantly higher than group IV at 95 per cent level

Peptic activity—The results of peptic activity estimations of the gastric juice are shown in Table I and Fig 5 Enough gastric juice was not available for estimation of peptic activity in groups I and II The mean value of peptic activity was 0 42 X 10³ and 0 18 X 10³ P. U. in groups III and IV respectively The difference in peptic activity is significantly higher in group III than group IV at 95 per cent level

Discussion

Gray and Ramsey (1956) pointed out that under normal circumstances the stomach was under partial control of the adrenal cortex and required normal adrenocortical function for peptic activity The diminution of gastric pepsinogen in normal adrenalectomized rats in the present experiments indicates that there may be a direct effect of adrenocorticoids in the formation of gastric pepsinogen or on the control on the ability of the peptic cells to synthesize pepsinogen

Pyloric obstruction in adrenal intact rats brought about depletion of mucosal pepsinogen and shrinkage of the peptic cells. Peptic activity in the gastric juice was also increased which showed a positive relationship with the increased degree of gastric ulceration This confirmed the findings of Singh, Zaidi and Balkrishna (1958) who showed that pepsinogen in pylorus ligated rats was significantly lower than in the normal, and increased peptic activity was directly proportional to the degree of peptic ulceration Gray *et al* (1957) suggested that under conditions of stress the stomach was more directly under the stimulatory control of the adrenocorticoids through hypothalamic-pituitary-adrenal axis. Pyloric obstruction in adrenal intact rats, by process of stress, possibly brought the stomach under the direct stimulatory control of adrenals and thus depleted more pepsinogen than normally formed

In the present experiments adrenalectomy significantly lowered the volume of gastric juice as well as free acid and further marked inhibition of peptic activity. The cytological

ACKNOWLEDGEMENTS

Thanks are due to Dr. B Mukerji, F. N. I for his interest in this work The authors are grateful to Mr M M Soni and Mr. Mulkraj for their technical assistance.

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DEMONSTRATION OF THE EXISTENCE OF PRECORTICOTROPIN—A PRECURSOR OF CORTICOTROPIN (ACTH) IN THE OXY ANTERIOR PITUITARY GLAND

by P R Dasgupta

Central Drug Research Institute Lucknow

In addition to ACTH (adrenocor...
called AW-factor causing only ...
adrenals of hypophysectomized ...
and Dixon *et al* (1951, 1953) to be present in the ox pituitary tissue Stack-Dunne ... (1951) Young (1954) and Cater and Stack-Dunne ...

... extracts of activity detectable in Sayers test was ... virtual or complete absence ... fresh ox anterior pituitary tissue contained much corticotropin extractable by acid-acetone

The original aim of the present ...
AW-factor present ...
to be devoid of ...

It is intended to describe in this paper only those ... corticotropin ... trate the existence in the ox anterior pituitary g... of corticotropin The position of AW-facto... subject matter of a separate communication

Materials & Methods

(1) *Rats*—Male albino rats, 8-12 weeks old kept in a constant environment were used. Adult female rats were occasional choice

(2) *Crude sterile alkal...*
30-60 minutes after killir...
extracted according to ...
will be referred to here ...

1 gm gland = 4 mls FGS
= 60—80 mgm. protein

(3) *Crude ACTH from FGS*—A certain volume of FGS was dried while frozen The solid obtained was treated with hydrochloric acid and acetone according to the method of Lyons (1937) and the extracted material corresponds to the L-fraction of Dixon and Stack-Dunne (1955)

(4) "*Precorticotropin*" - *Extract*—Fresh ox anterior pituitary lobes were dissected, minced, dried by filter paper, promptly weighed and then homogenized with glass homogenizer in chilled (4°c) 0 9 per cent sodium chloride solution The homogenate (20 mgm dry tissue/ml solvent) was spun (18 000 g) for 30 minutes The clear reddish supernatant fluid (pH 6 5 to 6 7) which contained the "Precorticotropin" was stored in the cool chamber (0°-4°C)

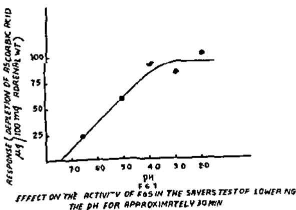
(5) *Bioassay*—The ascorbic acid-depletion method of Sayers *et al* (1948) was used with minor modifications The positive response is indicated by a fall of adrenals ascorbic acid-level (in µg/100 mg tissue) caused by an intravenous injection of the test material (0 25 ml/100 gm body weight)

Results

Sayers test of crude corticotropin extracted from FGS

Table 1 shows the adrenal ascorbic acid depleting activity of crude corticotropin extracted from FGS as compared with the activities of a standard ACTH preparation (Armour 84-85 H - kindly supplied by Armour Laboratories, Ltd, U S A), of the original FGS and of FGS at *pH 3 0* It is found that although the original untreated FGS possesses virtually no adrenal ascorbic acid-depleting activity yet the crude corticotropin extracted from it is active Further, activity appears in the FGS when its *p*H is adjusted to *p*H 3 0 by adding Hcl (2N) The reason for changing the *p*H of the original FGS to *p*H 3 is that in the Sayers assay the test material is administered after dissolving it in a trace-acetic acid-containing 0 9 per cent sodium chloride solution whose *p*H is 3

Effect on the Sayers activity of FGS of lowering the pH



FG 1
EFFECT ON THE ACTIVITY OF FGS IN THE SAYERS TEST OF LOWERING THE pH FOR APPROXIMATELY 30 MIN

TABLE 1

Sayers activity of the crude corticotropin extracted from FGS
(Figures within parenthesis indicate total number of animals minus sign indicates positive response)

	Change of adrenal ascorbic acid levels (in µg/100mg adrenal weight) produced by			
	Armour 84-85 H (0 5 m tu per ml)	Original FGS (pH 8 5)	Crude ACTH (2 mg per ml)	FGS at pH 3 (20 mg protein per ml)
Mean response ± standard error of the mean	−46 ± 5 83(3)	−10 ± 12 4(4)	−101 ± 19 8(3)	−72 ± 19 6(3)

TABLE 2

Effect on the Sayers activity of FGS of lowering the pH and then incubating at 37° for 1 hr (Figures within parentheses indicate total number of animal minus sign indicates positive responses)

	Change of adrenal ascorbic acid levels (µg /100 mg adrenal weight) by—							
	Original FGS (pH 8 5)		FGS incubated at 37° for 1 hr after adjusting the pH to —					
	Incubated	Not incubated	7	6	5	4	3	2
Mean ponse ± S E M	−5 ± 1 0(2)	+16 9 1(4)	+10 ± 13 5(3)	−18 + 11 4(3)	−44 ± 9 3(3)	−74 ± 6 1(3)	−68 ± 10 0(3)	−86 ± 13 2(3)

S E M = Standard error of the mean

Effect on the Sayers activity of FGS of addition of urea

Table 3 shows the effect of addition of urea on the activity of FGS as compared with the
was free
pink sol
amount ot crystalline urea (6 6 M) when a clear transparent solution was obtained

It is clear that Sayers activity appears in the FGS after addition of urea to it (Table 3).

TABLE 3

Sayers activity of FGS treated with urea (6 6M) (Minus sign indicates positive responses figures within parentheses indicate total number of animals)

Change of adrenal ascorbic acid content (μgm /100 mgm adrenal tissue weight) by FGS after various treatments —	
TREATMENT	Mean response ± S E M (*)
None i e original FGS	—19 ± 8 3 (3)
Adjustment to pH 3	*—35 ± 6 0 (3)*
Urea (6 6 m)	—66 ± 3 6 (4)
Blank 6 6m urea solution only	+11 ± 4 2 (3)

* S E M = Standard error of the mean

Sayers activity of "Precorticotropin" extract

Table 4 shows the ascorbic acid-depleting activity of the "Precorticotropin' - extracts (see page 3 for preparation) before and after treatments with various activating agents

Effect of Sodium chloride on "Precorticotropin"

Supernatants were obtained in the identical way as in the previous experiment out of a fresh pituitary-homogenates prepared in 0 5 per cent, 0 9 per cent and in 5 0 per cent sodium chloride solution without adjustment of *p*H The adrenal ascorbic acid-depleting activity of such supernatants was determined Further, the activity of the supernatants in 0 9 per cent and in 0 5 per cent sodium chloride solutions was determined separately after adding fresh quantity of sodium chloride to it such that its final concentration was 5 0 per cent

The results have been summarised in Table 5 It appears that sodium chloride also is capable of modifying the native precorticotropin into the material active in the Sayers test

Effect on "Precorticotropin" content of pituitary of acetone treatment and of repeated freezing and thawing

A similar experiment (Table 5) was conducted using supernatants of homogenates prepared not from fresh untreated glands but from glands which were initially subjected

Sayers act ty of tl e supernatant flu d of tl e hon ogena e of f e h ox a t r p u ary t sue
(F gures w thin parentheses nd cate total nun ber of an n als min ss gn n l cates posit ve respo se)

Fresh ox anterior p tu tary t ssue hon ogenate (o mg per ml) d v ded 1 to 2 port or s

Po n I Spu

Sol d tel omog 1 sed div ded o two p r ons

Port n I d lu ed (5n g pr cin ml) (*Solu on B*)
M R SEM = —1 4 13 6(4)

P o II adju ed to pH d l ed (5 mg tissue n l) (*Sol on C*)
M R SEM = —138 4

Supe nata d v de l 1 o 3 port o s

P d lu ed (5 b P o e l l (*Sol n D*)
M R SEM 1 8 o(7

II adj ust d t pH d l d (15 g prot l (*S l u l*)
M R SEM —84 8 o(9)

P o III urea added (6 6 M) d lute l (15mg prote ml) (*Sol on F*)
M R S I M = —61 + 15 o(4)

Po t on II d luted 10-f ld (*Sol t o A*)
M R ± S E M = —110 ± 7 5(5)

N B 1) D lut or s were o-f ld each ca e ma le w th o 9 per ce t s d u chl r le sol t o
2) M R —Mean respo se
3) S E M —Standard error of the n ea

TABLE 5

Effect of Sodium chloride on Precorticotropin

	Change of adrenal ascorbic acid (ug/100 mg adrenal weight) produced by precorticotropin —extract made in sodium chloride solution of various concentrations							
	5 0% NaCl (1 ml extract = 1 0 mg N_2)		0 9% NaCl (1 ml extract = 0 7 mg N_2)			0 5% NaCl (1 ml extract = 0 5 mg N_2)		
	Untreated & diluted	Adjusted to pH 3 & diluted	Untreated & diluted	Adjusted to pH 3 & diluted	Treated with NaCl & diluted	Untreated & diluted	Adjusted to pH 3 & diluted	Treated with NaCl & diluted
M R. ± S E M	−48±21 0(3)	−56±7 5(3)	−4±4 0(2)	−82±26 0(2)	−50±7 2(4)	−8±7 7(3)	−69±9 4(3)	−62±8 3(4)

* — Requisite amount of sodium chloride was added to the extract such that the final concentration was 5 per cent the extract was then incubated at 37°c for ½ hr before testing

N B
1) In each case the dilution was 20-fold and was made with distilled water
2) Figures within parentheses indicate the total number of animals taken
3) Minus sign indicates positive response
4) S E M —Standard error of the mean
5) M R —Mean response

either to (1) drying with acetone, or to (2) repeated freezing and thawing Table 6

DISCUSSION

is adjusted to 3 0 (Table 1) The reason for including FGS adjusted to pH 3 0 as one of the test materials (Table I) is that in Sayers assays done routinely by us the test materials are dissolved in 0 9 per cent sodium chloride solution containing acetic acid (0 25 per cent) pH of which is 3 0 This latter finding should naturally prompt one to study *the influence of pH on the unknown process associated with the production of Sayers* active material in the FGS However, there appears to be no such effect of pH change on the production of Sayers activity in FGS (Table I, Fig 1) which could be regarded as optimal indicating the involvement of enzymes in the process It is of interest to

Sayers activity appears in FGS when its pH is brought just to the acid side (pH 6 0) and the virtual lack of response at pH's 6 0 and 5 0 is due to the fact as observed by Geschwind

Here it can be argued that the absence of activity in the Sayers test of the crude alkaline *extract is due to the presence of proteolytic enzymes in such extracts which affect* material active in Sayers test much more adversely in the alkaline side than in the acid side That this, however, is not the case would appear from what follows

Since some biological substances of neutral nature such as urea have got profound actions upon the native structure of proteins the effect of urea on the above principle of FGS was studied Interestingly enough, the principle inactive in Sayers test has been changed into the active form (Table 3) in the presence of urea also

treatment required that the change brought about in FGS is not of drastic nature, and pertains rather to the internal condition of the above principle *than to its external.*

The question now arises whether this new principle detected in the FGS is a native substance of the pituitary gland or an artifact of preparation One way of answering

this question would be to demonstrate the presence of the principle in an extract prepared from very fresh pituitary glands under such mild conditions which preclude the chance [illegible]

above conditions Moreover, since this principle is present also in FGS it appears to be stable indefinitely at pH 7-9 It should be recalled that the activity of corticotropin is lost in presence of alkali Does the pituitary gland synthesize the release solely this principle into blood where it changes rapidly into corticotropin under the conditions of stress? Is then the corticotropin obtained from pituitary an artifact of preparation? However, this does not appear to be the case In the fresh homogenate of anterior pituitary tissue only a portion of the total corticotropin content is present as precorticotropin and is ineffective in the Sayers test (Table 4) It is possible that the conditions of killing the animals from which the pituitary glands are obtained, or the time which elapses between the death of the animal and the preparation of the homogenate, may affect the content of precorticotropin of the glands It is also possible that the pituitary elaborates and releases a mixture of corticotropin and precorticotropin in a fixed ratio which is maintained by breakdown (or otherwise) of the latter into corticotropin (homoeostatis)? It needs further investigation to answer these questions What is clear at present is that the ox anterior pituitary gland contains a principle which though inactive by itself in the Sayers test is convertible into a material active in this respect It is, therefore, proposed that this pituitary principle acting like a precursor of corticotropin is as designated as "Precorticotropin"

It was now decided to try extraction of fresh pituitary tissue for precorticotropin [illegible]

[illegible]

that drying of tissues with acetone or subjecting it to repeated freezing and thawing (5 times) prior to extraction with 0 9 per cent sodium chloride produced similar effects

TABLE 6

Activities in Sayers test of the Supernatants obtained from homogenates of (1) acetone dried (2) frozen ox anterior pituitary tissue in 0 9% NaCl solution (Figures within parentheses indicate total number of animals, minus sign indicates positive response)

	Change of adrenal ascorbic acid level (μgm /100 mgm adrenal weight) by-			
	Supernatant from acetone dried gland homogenate		Supernatant from frozen gland homogenate	
	Untreated & diluted 10-fold	Treated to pH3 and diluted 10-fold	Untreated & diluted 20-fold	Treated to pH3 and diluted 20-fold
Mean Response ± S E M	—127±9 0(6)	—139±16 3(7)	—70±10 8(4)	—98±1 4(4)

S E M = Standard error of the mean

The observation made in presence of acetone is in agreement with that by Carlisle (1958) in respect of a crustacean chrom-activator

Whatever may be the nature of actions of these treatments upon precorticotropin it is unlikely that the change brought about by PH-lowering urea and sodium chloride would go beyond breaking of hydrogen bonds of the native material Hall (1944) Mettes and Turner (1948) and Carlisle (1957) obtained highly lactogenic material from pituitary extracts which by itself was inactive in this respect when tested in rabbits In fact, Carlisle (1957) believes that the conventional prolactin (luteotropin) is really an artifact of preparation

It seems quite probable that the presence of precorticotropin in a crude alkaline extract of ox pituitary tissue accounts for the striking discrepencies between AA-activity and AW-activity of such extracts Furthermore this demonstration of the presence of precorticotropin in the pituitary tissue can pertinently be expected to shed some new light on the probable relationship among (i) the factor of pregnant mare serum described by Golla and Reiss (1941-42) (ii) the "activable corticotropin" of Addisonian blood reported by Moruzzi *et al* (1954) and (iii) the adrenal weight increasing factor of plasma of patients with Cushing's Syndrome due to bilateral adrenal hyperplasia and of pregnant women lately described by Jailer *et al* (1957) It is tempting to speculate that our precorticotropin is identifiable with the "activable corticotropin" of Moruzzi *et al* (1954) It is probable that our precorticotropin is present in blood and that the high level of sodium and chloride ions in the blood of patients with Cushing's Syndrome converts precorticotropin into corticotropin as soon as the former enters the circulation and that the high level of urea (another of our activating agents) is rendered inoperative in the blood of Addisonians due to the high level of potassium which however is low in the blood of patients with Cushing's Syndrome

ACKNOWLEDGEMENTS

A note on some of the earlier findings made in the investigation described in this paper was published in *Nature* (**182**, 32 1958) jointly with Professor F G Young F R S who suggested the problem and to whom the author is greatly indebted The author is grateful also to Dr B Mukerji, Director Central Drug Research Institute Lucknow for allowing him to continue the study at C D R I Thanks are due to Miss M B Thomas of the Biochemistry Department University of Cambridge and to Sri K Narain of the Endocrinology Section C D R I Lucknow for their skilled assistance

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HISTOCHEMICAL STUDY OF ADRENAL CORTICAL ACTIVITY IN TOAD (BUFO MELANOSTICTUS)

by CHANDICHARAN DEB and MRITYUNJOY MUKHERJI

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The study of comparative endocrinology have gained importance in recent years The adrenal gland in toad is such that it can not be separated from the kidney, making biochemical estimations impracticable So adrenal cortical activity in this species under various conditions was studied by histochemical methods

Seasonal Variation Mukherji and Deb (1959) have studied seasonal variation in neutral lipids, unsaturated lipids, plasmalogen and cholesterol in toads adrenal during hibernation (February), post hibernation (April), breeding season (August) and pre hibernation (November) Maximum amount of sudan positive lipids and cholesterol was observed during February and minimum during April In August and November there was a gradual rise from April The unsaturated lipids and plasmalogen also reached their maximum during February and the minimum having been attained during April In most of the species the adrenal cortex was found to contain a high amount of cholesterol (Cowte and Magee 1934) Injection of A C T H in rats and guinea pigs lowered both ascorbic acid and cholesterol (Sayers *et al* 1946) In toad, however we failed to detect any histochemically demonstrable ascorbic acid in adrenal gland Recently Elton Zarrow and Zarrow (1959) have observed chemically a very low value of ascorbic acid in toad's adrenal compared to other species So estimation of ascorbic acid was not included in the present investigation. Sayers and his co-workers (1950) have observed an intimate relationship between the density of sudanophilic material and the concentration of cholesterol in adrenals He has also correlated distribution of neutral lipids in adrenals with its secretory activity and have concluded that in general lipid depletion denoted hyperactivity and lipid accumulation hypoactivity of the gland Our findings of increased sudanophilia and cholesterol during November and February thus suggested hypoactivity of the gland, an increased activity as observed by reduced sudan positive substance have been observed during April-August Maximum amount of plasmal has been observed during February and minimum during April Albert and Leblond (1946) and also Dempsey and Wislocki (1944) have suggested that plasmal reaction like phenylhydrazine could be used for detection of ketosteroids Accordingly maximum accumulation of ketosteroids in adrenals during February indicated a non functional accumulation of it Our observation of hypofunction of adrenals during hibernation was in confirmatory with the observation of Lyman and Chatfield (1955) who also have observed a quiescent adrenal in golden hamsters during hibernation Experiments are in progress to observe if this hypofunction is due to lowered A C T H production

Effect of acute dehydration The effect of stress produced by acute water deprivation for 48 hours on toad's adrenals were studied by Deb and Mukherji (1959) To observe the effect of seasonal variation on adrenal response to stress the experiment was carried out during hibernation and non hibernation Changes in neutral lipid, unsaturated lipid, plasmalogen and cholesterol were detected histochemically In the nonhibernating toad's adrenal there was a depletion of all the four histochemically demonstrable substance compared to normal ones Toads were also subjected to similar stress during hibernation when there

was no alteration in the lipids Sayers and his co-workers (1944) have observed that when there was an increased demand for adrenal cortical hormone such as stress, there was a decrease in the cholesterol content adrenals Our findings of the fall in lipids including cholesterol might be due to similar reasons It has also been observed that in the absence of anterior pituitary the adrenal cholesterol did not change to stress (Patt *et al* 1948, Sayers *et al* 1945) Due possibly to blockade of pituitary adrenal axis in hibernating toad, no alteration in the lipids were observed by us

[illegible]

the cytoplasmic inclusions which were further depleted after 48 hours. Long (1947) [illegible]

[illegible] longed exposure has not yet been studied Elton, Zarrow and Zarrow (1959) have failed to observe any alteration in the cholesterol content of toad's adrenal on exposure to cold (0-5°C) for 1-4 hours The authors, however, have not maintained if they have used hibernating or non hibernating toad

It can be concluded that toad's adrenal also respond to different stress conditions, but only during non hibernating period.

Acknowledgement

Thanks are due to Prof P B Sen, Professor of Physiology, for his kind suggestions and encouragement

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Explanation of Plate VII

Fig 1 Adrenal, cholesterol, after 48 hours' dehydration, during August, Compare with Fig 5 x 96

Fig 2 Adrenal, neutral lipid during February Compare with Fig 3 x 96

Fig 3 Adrenal, neutral lipid, during April, Compare with Fig 2 x 96

Fig 4 Adrenal cholesterol during February Compare with Fig 5 x 96

Fig [illegible] Compare with Fig 4 x 96

Fig [illegible] Compare with Fig 7 x 96

Fig [illegible] mpare with Fig 6 x 96

Fig 8 Adrenal, neutral lipid after 48 hours dehydration, during April Compare with Fig 3 x 96

Fig 9 Adrenal, cholesterol, after "Cold shock for 3 hours", during August Compare with Fig 5 x 96

Fig 10 Adrenal, cholesterol, after "Cold shock for 48 hours", during August Compare with Fig 5 x 96.

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INVESTIGATIONS ON AUTOTRANSPLANTATION OF THE ADRENAL GLAND IN THE SPLEEN WITH SPECIAL REFERENCE TO PITUITARY-ADRENAL-RELATIONSHIPS

by A S. Krishna Murthy and M B. Sahasrabudhe

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In connection with a research project under the auspices of the I. C. M. R. for the "Development of a Histochemical Method for the Localisation of Adrenocorticotropic

probably get detoxicated and made physiologically inactive. This technique of autotransplantation of the adrenal into spleen has been employed in the present investigation to study its response on pituitary histology at different intervals of time.

In the present investigation Wistar rats of both the sexes and about eight to ten weeks of age were used Both the adrenals were removed under aether anaesthesia, and a piece of adrenal tissue introduced in the spleen with a trocar The animals were maintained on isotonic saline for about eight to ten days after operation Saline was later withdrawn and the animals were reverted on water Mortality rate was high due to operative trauma

The operated animals were divided into four groups. The first, second, third and

The changes in the graft and pituitary gland were as follows.

control animals

... from the foregoing, that autotransplantation of adrenal into spleen ... pituitary gland Basophils are supposed to ... tropic hormones (TSH), gonadotropic hormones (GH) and ACTH In the present investigation, however, it appears that cells associated with the production of ACTH were increased, as evidenced by the proliferation

of adrenal graft in the spleen TSH or GH however were not appreciably altered as the thyroid gland and gonads did not show any proliferation

DISCUSSION

Kar If I remember correctly Seligman and Rutenburg found that there was a difference in staining intensity with aerobic *vs* anaerobic incubation Dr De'Souza, what type of incubation did you use in your studies ?

De Souza It is correct that a difference in staining intensity under aerobic and anaerobic conditions was found in early studies where tetrazolium salts were mostly used We used aerobic incubation with nitro B T - a fast electron acceptor To my mind the difference is not very marked as the cytochrome system cannot compete as efficiently with nitro B T for the electron acceptance

K.. [illegible]

[illegible] action at the cellular level The recent attempts by workers like Glick to make these techniques more refined and quantitative are proving to be extremely valuable

Kar [illegible]

Singh [illegible]

Prasad

Singh No

Malaviya Dr Singh, your experiments are well planned and your results are interesting But, I feel that it is a little too much to draw any resemblance with gastric ulcer as found in human [illegible]

LIST OF THOSE PARTICIPATING IN OR ATTENDING THE SYMPOSIUM ON "THYRO-GONAD-ADRENAL-PITUITARY RELATIONSHIPS"—NEW DELHI OCTOBER 2 3 & 4 1959

A Ahuja	[illegible]
K N Bagchi	[illegible]
S Banerjee	[illegible]
U K Banik	[illegible] ntal Medicine Calcutta
B Chowdhury	Defence Science Organisation Delhi
R R Chowdhury	All India Institute of Medical Sciences, New Delhi
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P R Das Gupta	Central Drug Research Institute Lucknow
B B Dixit	All India Institute of Medical Sciences New Delhi
E J De Souza	Topiwala National Medical College Bombay
A B Kar	[illegible]
A S Krishna Murthy	[illegible]
B K Malaviya	[illegible]
B Mukerji	[illegible]
S R Mukherjee	[illegible]
M R N Prasad	University of Delhi Delhi
K Pal	Indian Veterinary Research Institute Izatnagar
M B Sahasrabudhe	Atomic Energy Research Establishment Bombay
P N Shah	Indian Cancer Research Centre Bombay
G B Singh	Central Drug Research Institute Lucknow
V Subrahmanian	Central Food Technological Research Institute Mysore
V N Talawar	All India Institute of Medical Sciences, New Delhi